Blood Levels of Homocysteine and Increased Risks of Cardiovascular Disease

Causal or Casual?

William G. Christen, ScD; Umed A. Ajani, MBBS; Robert J. Glynn, ScD; Charles H. Hennekens, MD

Background: Accumulating data from epidemiological studies suggest that individuals with elevated blood levels of homocysteine have increased risks of cardiovascular disease. We reviewed the currently available evidence of an association between homocysteine and cardiovascular disease and examined whether the strength of the evidence varies according to study design.

Methods: We used a computerized MEDLINE literature search, 1966 through September 1998, to identify all epidemiological studies that examined the relationship of homocysteine level with risks of coronary heart disease or cerebrovascular disease. Two measures of plasma homocysteine level and its association with risk of cardiovascular disease were extracted: mean homocysteine level in cases and controls, and relative risk of cardiovascular disease for elevated homocysteine level.

Results: A total of 43 studies were reviewed. Most cross-sectional and case-control studies indicated higher mean homocysteine levels (either fasting or after methionine load) and/or a greater frequency of elevated homocysteine level in persons with cardiovascular disease as compared with persons without cardiovascular disease. Results of most prospective studies, however, indicated smaller or no association. The few prospective studies that reported a positive association between homocysteine level and risks of cardiovascular disease included patients with preexisting vascular disease.

Conclusions: In contrast to cross-sectional and case-control studies, results of prospective studies indicated less or no predictive ability for plasma homocysteine in cardiovascular disease. Instead, elevated homocysteine level may be an acute-phase reactant that is predominantly a marker of atherogenesis, or a consequence of other factors more closely linked to risks of cardiovascular disease. Randomized trials are necessary to test reliably whether lowering homocysteine levels will decrease risks of cardiovascular disease.

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Homocystinuria is a rare autosomal recessive condition usually resulting from homozygous deficiency of cystathionine β-synthase, an enzyme required in methionine metabolism for the conversion of homocysteine to cystathionine. Patients with homocystinuria commonly have fasting plasma total homocysteine (sum of free plus protein-bound forms) levels exceeding 250 µmol/L, compared with a reference range of 5 to 15 µmol/L in healthy subjects, and are prone to premature atherosclerosis and thromboembolism of the extracranial and intracranial cerebral arteries and veins, the coronary arteries, and the peripheral arteries and veins. While other enzymatic defects can also produce homocystinuria, the observation 3 decades ago that vascular disease was common regardless of the source of the defect suggested that homocysteine may be responsible for the vascular abnormality. Plausible mechanisms to mediate a deleterious effect of high homocysteine level include vascular endothelial dysfunction, promotion of oxidation of low-density lipoprotein cholesterol, vascular smooth cell proliferation, and coagulation abnormalities.

Accumulating data from epidemiological studies suggested that individuals with even moderately elevated levels of homocysteine (eg, fasting blood levels exceeding approximately 16 µmol/L) have small to moderate increased risks of cardiovascular disease (CVD). Elevated homocysteine levels are common in the general population; 21% of elderly participants in the Framingham Study had plasma homocysteine levels exceeding 15.8 µmol/L. These can result from heterozygous deficiency of cystathionine β-synthase or methylenetetrahydrofolate reductase (an enzyme involved in remethylation of homocysteine to methionine), or from suboptimal intake of nutritional factors (folate, vitamin B6, vitamin B12) required for homocysteine metabolism. Whether high homocysteine level is a cause of CVD is not yet clear.
A recent review that combined available data from cross-sectional, case-control, and a limited number of prospective studies concluded that an elevated level of homocysteine was an independent risk factor for coronary heart disease (CHD), cerebrovascular disease, and peripheral vascular disease. More recent prospective data, however, tend to show little or no association between homocysteine levels and subsequent CVD. Nevertheless, despite these more recent and apparently conflicting data, enthusiasm continues to be expressed for screening of homocysteine level as a regular component of CVD prevention, largely because the levels can be easily reduced by administration of pyridoxine hydrochloride, cyanocobalamine, and folic acid.

In this review we present the currently available evidence regarding homocysteine and CVD. We also consider the strengths and limitations of completed cross-sectional, case-control, and prospective studies, and suggest future directions.

**METHODS**

We used a computerized MEDLINE English-language literature search, 1966 through September 1998, to identify all epidemiological studies that examined the relationship of homocysteine level with risks of CHD or cerebrovascular disease. We also searched reference lists of all identified articles for additional relevant studies.

**Criteria for Inclusion**

There were 2 criteria for inclusion of studies in the review: reporting of data for plasma homocysteine concentration and assessment of CHD or cerebrovascular disease status. A total of 43 studies were identified that reported original data on plasma homocysteine concentration and CVD risks. Studies of CHD included the end points of fatal and nonfatal myocardial infarction (MI) and angiographically confirmed occlusion; studies of cerebrovascular disease included ischemic and hemorrhagic stroke and carotid atherosclerosis. Some studies examined multiple CVD end points (eg, MI and stroke), and these data are presented separately according to CVD type in this review. If data in the original report were not presented separately for specific CVD end points, that study's overall results were included in the CVD category most frequently represented in the report (CHD or cerebrovascular disease). Studies conducted within a dialysis population (see review supplements) or in patients with end-stage renal disease were not included in this review. We also excluded 4 studies because of incomplete information, questionable homocysteine values, or cases selected on genetic or familial factors presumed to be related to homocysteine level.

Studies were categorized as cross-sectional, case-control, or prospective. Studies in which homocysteine was measured at or after the identification of cases were considered either cross-sectional (if members of a defined population were examined for the presence or absence of the CVD end point) or case-control (if a distinct control group was identified as a standard of comparison). Studies in which homocysteine was measured before the identification of cases were considered prospective and included prospective cohort studies and case-control studies nested within a prospective cohort. In all study designs, persons with the CVD end point were designated as cases and those without the end point were designated as controls.

Several components of homocysteine have been measured in the included reports, and these components are indicated in the tables. Homocysteine is a thiol-containing amino acid formed from the metabolism of methionine and is oxidized in plasma to the disulfide homocystine (Hcy-Hcy in tables) and to the mixed disulfide homocysteine (Hcy-Cys in tables). These 2 compounds occur naturally in plasma in both free and protein-bound forms and are collectively referred to as plasma total homocysteine (tHcy in tables).

**Data Presentation**

Results for each study are presented by means of the following measures of plasma homocysteine level and its association with risk of CVD.

1. **Mean homocysteine**: This includes mean level of homocysteine (micromoles per liter) (basal, fasting, or post–methionine challenge) in cases and controls, and the associated P value. In studies where overall mean level of homocysteine for cases and controls was not presented (eg, several studies presenting only sex-specific values), we estimated the overall mean for cases and controls from the reported data and conducted statistical comparisons using a pooled estimate of the variance. Because the raw data were not available, more appropriate nonparametric tests (Wilcoxon rank sum) could not be used for these comparisons. However, many studies reported the use of parametric tests (t tests) on loge-transformed data to compare homocysteine levels in cases and controls, and several studies indicated that the results for nontransformed and loge-transformed data were similar.

2. **Elevated homocysteine level**: For each study, we indicate the definition of elevated homocysteine level used by the investigators, the relative risk (RR) of CVD and 95% confidence interval (95% CI) associated with elevated levels of homocysteine, and the covariates controlled for by matching or in analyses. When the original report presented only the percentage of cases and controls that met the definition of elevated homocysteine level, we estimated the RR of CVD by computing the odds ratio and estimated the 95% CI by using the values of the 2 × 2 table according to Woolf's method. If the 2 × 2 table contained a value of 0 in any one of the cells, the odds ratio was estimated by adding 0.5 to each cell.

We did not calculate a quantitative summary estimate of the association between elevated homocysteine levels and CVD because of important differences in the populations studied, including differences in the range of homocysteine values, the cutoff points used to define elevated homocysteine level, and the differing degree of control of confounding in the various studies, all of which are observational, not randomized.
Epidemiological Evidence

Cross-Sectional and Case-Control Studies

Cross-sectional and case-control studies generally support an association of elevated homocysteine levels with increased risks of CVD. Coronary Heart Disease. There have been 5 cross-sectional studies of homocysteine and CHD35-39 (Table 1 and Figure 1). In general, the determination of CHD has been based on angiographic evidence of greater than 50% occlusion of at least 1 coronary artery. Blood samples were collected around the time of CHD determination. Four cross-sectional studies reported mean homocysteine levels of 22, 24, 24, and 22 µmol/L, respectively. Four cross-sectional studies reported mean homocysteine levels of 22, 24, 24, and 22 µmol/L, respectively.

Table 1. Characteristics of 31 Reports of Homocysteine and Coronary Heart Disease

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Age, y</th>
<th>Sample Size</th>
<th>Species Measured</th>
<th>Mean Homocysteine, µmol/L</th>
<th>Cases Controls P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilcken and Wilcken,1976</td>
<td>&lt;50</td>
<td>25 Cases 22 Controls</td>
<td>4-h PML Hcy-Cys</td>
<td>22</td>
<td>NG NG ...</td>
</tr>
<tr>
<td>Murphy-Chutorian et al,1985</td>
<td>21-65</td>
<td>99 Cases 39 Controls</td>
<td>Fasting and 6-h PML Hcy-Hcy</td>
<td>0.03 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Kang et al,1986</td>
<td>&lt;69</td>
<td>241 Cases 202 Controls</td>
<td>Fasting protein-bound Hcy</td>
<td>5.5 4.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Malinow et al,1990</td>
<td>Mean, 62</td>
<td>99 Cases 259 Controls</td>
<td>Basal Hcy</td>
<td>13.0 10.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Murphy-Chutorian and Alderman,1994</td>
<td>17-80</td>
<td>80 Cases 22 Controls</td>
<td>6-h PML Hcy-Hcy</td>
<td>0.76 0.40</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Wilcken et al,1983</td>
<td>&lt;50</td>
<td>20 Cases 20 Controls</td>
<td>Fasting and 4-h PML Hcy-Hcy</td>
<td>3.6 3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Boers et al,1985</td>
<td>50</td>
<td>25 Cases 40 Controls</td>
<td>Peak PML Hcy-Hcy and Hcy-Cys</td>
<td>13.7 12.9</td>
<td>P</td>
</tr>
<tr>
<td>Israelsson et al,1988</td>
<td>48-58</td>
<td>21 Cases 36 Controls</td>
<td>Fasting Hcy</td>
<td>16.4 13.5</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Genest et al,1990</td>
<td>&lt;60</td>
<td>170 Cases 255 Controls</td>
<td>Fasting Hcy</td>
<td>13.7 10.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Clarke et al,1991</td>
<td>&lt;55</td>
<td>60 Cases 27 Controls</td>
<td>Peak PML Hcy-Hcy and Hcy-Cys</td>
<td>18.7 13.4</td>
<td>P</td>
</tr>
<tr>
<td>Ubbink et al,1991</td>
<td>Mean, 55</td>
<td>163 Cases 195 Controls</td>
<td>Fasting Hcy</td>
<td>16.2 13.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dudman et al,1993</td>
<td>&lt;53</td>
<td>62 Cases 56 Controls</td>
<td>4-h and 8-h PML Hcy-Hcy and Hcy-Cys</td>
<td>13.5 11.9 (G)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pancharuniti et al,1994</td>
<td>30-50</td>
<td>101 Cases 108 Controls</td>
<td>Fasting Hcy</td>
<td>13.5 11.9 (G)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>von Eckardstein et al,1994</td>
<td>36-65</td>
<td>199 Cases 156 Controls</td>
<td>Basal Hcy</td>
<td>8.9 7.8 (G)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Wu et al,1994</td>
<td>&lt;65</td>
<td>170 Cases 168 Controls</td>
<td>Fasting Hcy</td>
<td>13.4 10.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dalery et al,1995</td>
<td>&lt;60</td>
<td>150 Cases 584 Controls</td>
<td>Fasting Hcy</td>
<td>11.7 9.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Langdren et al,1995</td>
<td>28-81</td>
<td>68 Cases 80 Controls</td>
<td>Basal Hcy</td>
<td>13.9 12.3</td>
<td>NS</td>
</tr>
<tr>
<td>Robinson et al,1995</td>
<td>Mean, 62</td>
<td>304 Cases 231 Controls</td>
<td>Fasting Hcy</td>
<td>14.4 10.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Gallagher et al,1996</td>
<td>Mean, 49 (cases), 48 (controls), &lt;76</td>
<td>71 Cases 92 Controls</td>
<td>Fasting and 6-h PML Hcy</td>
<td>NG NG ...</td>
<td></td>
</tr>
<tr>
<td>Verhoef et al,1996</td>
<td>Mean, 55</td>
<td>130 Cases 118 Controls</td>
<td>Fasting Hcy</td>
<td>10.2 9.1 (G)</td>
<td>.006</td>
</tr>
<tr>
<td>Graham et al,1997</td>
<td>&lt;60</td>
<td>383 Cases 800 Controls</td>
<td>Fasting and 6-h PML Hcy</td>
<td>11.2 9.7 (G)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Schwartz et al,1997</td>
<td>18-44</td>
<td>79 Cases 368 Controls</td>
<td>Basal Hcy</td>
<td>13.4 11.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Verhoef et al,1997</td>
<td>25-65</td>
<td>131 Cases 189 Controls</td>
<td>Fasting and 6-h PML Hcy</td>
<td>13.5 12.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

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Elevated Homocysteine

<table>
<thead>
<tr>
<th>Definition</th>
<th>RR (95% CI)</th>
<th>Adjusted Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML Hcy-Cys $\geq 10$ µmol/L</td>
<td>8.2 (0.9-72.8)</td>
<td>. . .</td>
</tr>
<tr>
<td>PML Hcy-Hcy $&gt;1.19$ µmol/L (95th percentile for controls)</td>
<td>7.3 (0.9-57.3)</td>
<td>. . .</td>
</tr>
<tr>
<td>NG</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Basal tHcy $&gt;95$th percentile for same-sex controls</td>
<td>3.7 (1.7-8.2)</td>
<td>. . .</td>
</tr>
<tr>
<td>PML Hcy-Hcy $&gt;1.9$ µmol/L</td>
<td>7.4 (0.4-131.5)</td>
<td>. . .</td>
</tr>
</tbody>
</table>

(continued)

Homocysteine levels for persons with and without CHD. In 3 of these studies, mean homocysteine level was higher (approximately 30%-90% higher) in persons with CHD, while the other study found no difference in mean homocysteine level in those with and those without CHD. All 4 cross-sectional studies that defined elevated homocysteine level indicated markedly increased odds of CHD in persons with high homocysteine levels, although the CIs were wide, reflecting the generally small sample sizes in these studies.

There have been 18 case-control studies of homocysteine and CHD (Table 1 and Figure 1). Case groups have generally consisted of persons with a recent
or angiographic evidence of CHD.* In most studies, blood samples were collected at least several months after the acute event.

Fifteen studies examined mean homocysteine levels,† and all but 3 reported significantly higher homocysteine levels (typically 10%-30% higher), either fasting or after methionine load, in persons with CHD as compared with persons without CHD. Fifteen of 16 studies that compared proportions with elevated homocysteine levels indicated that the increase in risk was statistically significant at the P<.05 level. In the 16th study, levels of non–protein-bound homocysteine after methionine load were similar in patients with CHD (n = 25) and their sex- and menopause-matched controls.41

Cerebrovascular Disease. The only cross-sectional analysis of homocysteine level and cerebrovascular disease consisted of 1041 elderly asymptomatic subjects from the Framingham Heart Study and found that elevated plasma homocysteine levels were associated with a 2-fold increased risk of extracranial carotid artery stenosis40 (Table 2 and Figure 2).

Table 2. Characteristics of 31 Reports of Homocysteine and Coronary Heart Diseasea (cont)

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Age, y</th>
<th>Sample Size</th>
<th>Species Measured</th>
<th>Mean Homocysteine, µmol/L</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfthan et al, 1994</td>
<td>40-64</td>
<td>191 Cases, 269 Controls</td>
<td>Basal tHcy</td>
<td>9.6, 9.6</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arnesen et al, 1995</td>
<td>12-61</td>
<td>122 Cases, 478 Controls</td>
<td>Basal tHcy</td>
<td>12.7, 11.3</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chasan-Taber et al, 1996</td>
<td>40-84</td>
<td>333 Cases, 149 Cases</td>
<td>Basal tHcy</td>
<td>10.9, 10.4, 12.7</td>
<td>NS, NS, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verhoeof et al, 1997</td>
<td>40-84</td>
<td>149 Cases, 120 Cases</td>
<td>Basal tHcy</td>
<td>12.7, 12.9</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evans et al, 1997</td>
<td>35-57</td>
<td>240 Cases, 472 Controls</td>
<td>Basal tHcy</td>
<td>12.7</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nygard et al, 1997</td>
<td>Median, 62</td>
<td>587 Patients with CAD (64 deaths during follow-up)</td>
<td>Fasting tHcy</td>
<td>NG, NG</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wald et al, 1998</td>
<td>35-64</td>
<td>229 Cases, 1126 Controls</td>
<td>Fasting tHcy</td>
<td>13.1</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folsom et al, 1998</td>
<td>45-64</td>
<td>232 Cases, 537 Controls</td>
<td>Fasting tHcy</td>
<td>8.9</td>
<td>5.5 (G)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*RR indicates relative risk; CI, confidence interval; PML, post–methionine load test; Hcy-Cys, homocysteine-cysteine mixed disulfide; NG, not given; ellipses, not applicable; Hcy-Hcy, homocystine; P, PML; Basal, nonfasting; tHcy, total homocysteine; G, geometric mean; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; and CAD, coronary artery disease.

†Relative risk and 95% CI derived from original report based on percentage of cases and controls with elevated homocysteine.

‡Calculated as the twice the concentration of Hcy-Hcy plus the concentration of Hcy-Cys.

§Adjusted RR comparing highest and lowest quintiles.

Figure 2. Cerebrovascular Disease. The only cross-sectional analysis of homocysteine level and cerebrovascular disease consisted of 1041 elderly asymptomatic subjects from the Framingham Heart Study and found that elevated plasma homocysteine levels were associated with a 2-fold increased risk of extracranial carotid artery stenosis (Table 2 and Figure 2).

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Six reports were from case-control studies nested within a prospective cohort, and 1 was from a nested case-cohort study. Thus, blood samples were collected before the development of any cardiovascular (CV) event, and analyzed, in most cases, several years after collection.

Among 191 cases of MI, identified during 9 years of follow-up, and 269 controls matched for sex and age from a source population of 7424 Finnish men and women free of atherosclerotic disease at baseline, no statistically significant difference was found in mean homocysteine level or in the proportion of cases and controls with elevated homocysteine levels, defined as homocysteine level greater than the 95th percentile for same-sex controls (RR, 1.3; 95% CI, 0.6-2.4). In the Tromso health study of almost 22,000 men and women free of MI at baseline, mean homocysteine level for 122 participants who had a diagnosis of CHD or died suddenly after onset of chest pain during follow-up (mean, 4 years) was significantly higher than the level in 478 controls matched for age, sex, and number of
5.7 Elevated homocysteine levels were not defined in the study, but the adjusted RR for each 4-μmol/L increase in homocysteine of 1.3 (95% CI, 1.1-1.7) suggested a graded relationship between homocysteine and risk of MI. In this study, 11.5% of cases and 1% of controls reported a history of angina pectoris at baseline.

In an analysis of baseline blood specimens for 333 participants who experienced a first MI during 7.5 years of follow-up in the Physicians’ Health Study and 333 controls matched for age and smoking, plasma homocysteine levels above the 95th percentile of the control distribution were associated with an elevated risk of MI, as indicated by a relative risk of 1.3 (95% CI, 1.1-1.7) for each 4-μmol/L increase in homocysteine.

### Table 2. Characteristics of 17 Reports of Homocysteine and Cerebrovascular Disease

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Age, y</th>
<th>Sample Size</th>
<th>Species Measured</th>
<th>Mean Homocysteine, µmol/L</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selhub et al, 1995</td>
<td>67-96</td>
<td>1041Persons (38% with extracranial carotid artery stenosis ≥25%)</td>
<td>Basal tHcy</td>
<td>NG</td>
<td>NG</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>Brattstrom et al, 1984</td>
<td>34-63</td>
<td>19 Cases</td>
<td>Fasting and 4-h PML Hcy-Cys</td>
<td>5.1</td>
<td>3.5</td>
<td>&lt;.05</td>
<td></td>
</tr>
<tr>
<td>Boari et al, 1985</td>
<td>&lt;50</td>
<td>25 Cases</td>
<td>Peak PML Hcy-Hcy and Hcy-Cys</td>
<td>NG</td>
<td>NG</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>Araki et al, 1989</td>
<td>39-79</td>
<td>45 Cases</td>
<td>Fasting tHcy</td>
<td>13.1</td>
<td>8.6</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Brattstrom et al, 1990</td>
<td>24-63</td>
<td>18 Cases</td>
<td>Fasting and 4-h PML tHcy</td>
<td>13.2</td>
<td>11.0</td>
<td>&lt;.05</td>
<td></td>
</tr>
<tr>
<td>Coull et al, 1990</td>
<td>Mean, 67 (cases), 61 (controls)</td>
<td>41 Cases</td>
<td>Fasting tHcy</td>
<td>15.8</td>
<td>10.7</td>
<td>&lt;.001</td>
<td></td>
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<tr>
<td>Clarke et al, 1991</td>
<td>&lt;55</td>
<td>38 Cases</td>
<td>Peak PML Hcy-Hcy and Hcy-Cys</td>
<td>20.4</td>
<td>13.4 (P, G)</td>
<td>&lt;.05</td>
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<tr>
<td>Brattstrom et al, 1992</td>
<td>38-72</td>
<td>70 Cases</td>
<td>Fasting tHcy</td>
<td>16.8</td>
<td>11.9</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Dudman et al, 1993</td>
<td>&lt;61</td>
<td>51 Cases</td>
<td>4-h and 8-h PML Hcy-Hcy and Hcy-Cys</td>
<td>NG</td>
<td>NG</td>
<td>. . .</td>
<td></td>
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<tr>
<td>Malinow et al, 1993</td>
<td>45-64</td>
<td>287 Cases</td>
<td>Fasting tHcy</td>
<td>9.3</td>
<td>8.3</td>
<td>&lt;.001</td>
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<tr>
<td>Lindgren et al, 1995</td>
<td>51-98</td>
<td>162 Cases</td>
<td>Basal tHcy</td>
<td>13.4</td>
<td>13.8</td>
<td>NS</td>
<td></td>
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<tr>
<td>Kluitmans et al, 1996</td>
<td>13-75</td>
<td>58 Cases</td>
<td>Fasting and PML tHcy</td>
<td>14.1</td>
<td>12.5</td>
<td>&lt;.05</td>
<td></td>
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<tr>
<td>Graham et al, 1997</td>
<td>&lt;60</td>
<td>211 Cases</td>
<td>Fasting and 6-h PML tHcy</td>
<td>11.1</td>
<td>9.7 (G)</td>
<td>NG</td>
<td></td>
</tr>
<tr>
<td>Alfihan et al, 1994</td>
<td>40-64</td>
<td>74 Cases</td>
<td>Basal tHcy</td>
<td>10.3</td>
<td>9.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Verhoeof et al, 1994</td>
<td>40-84</td>
<td>109 Cases</td>
<td>Basal tHcy</td>
<td>11.1</td>
<td>10.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Perry et al, 1995</td>
<td>40-59</td>
<td>107 Cases</td>
<td>Basal tHcy</td>
<td>13.7</td>
<td>11.9 (G)</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Petri et al, 1996</td>
<td>Mean, 35</td>
<td>29 Cases among 337 SLE patients</td>
<td>Fasting tHcy</td>
<td>10.3</td>
<td>7.4</td>
<td>&lt;.01</td>
<td></td>
</tr>
</tbody>
</table>

4 RR indicates relative risk; CI, confidence interval; Basal, nonfasting; tHcy, total homocysteine; NG, not given; ellipses, not applicable; HDL, high-density lipoprotein; PML, post–methionine load test; P, PML; Hcy-Hcy, homocystine; Hcy-Cys, homocysteine-cysteine mixed disulfide; G, geometric mean; BMI, body mass index; and SLE, systemic lupus erythematosus.

5 Adjusted RR comparing highest and lowest quartiles.

6 Calculated as twice the concentration of Hcy-Hcy plus the concentration of Hcy-Cys.

7 Relative risk and 95% confidence interval derived from original report based on percentage of cases and controls with elevated homocysteine.

8 Mean for 45 normotensive controls and 45 hypertensive controls.

9 Adjusted RR comparing highest and lowest quintiles.

10 Median value.

11 P value for comparison of coronary heart disease cases and controls not given; P value for comparison of all vascular cases (coronary heart disease, cerebrovascular disease, peripheral vascular disease) and controls, <.001.

12 Adjusted RR comparing highest 5% vs the lower 90% of tHcy levels.

13 Relative risk and 95% confidence interval derived from original report based on percentage of cases and controls with elevated homocysteine.

14 Mean for 45 normotensive controls and 45 hypertensive controls.

15 Adjusted RR comparing highest and lowest quintiles.

16 Median value.

17 P value for comparison of coronary heart disease cases and controls not given; P value for comparison of all vascular cases (coronary heart disease, cerebrovascular disease, peripheral vascular disease) and controls, <.001.

18 Adjusted RR comparing highest 5% vs the lower 90% of tHcy levels.

19 Adjusted RR comparing tHcy levels greater than 15.3 µmol/L with levels less than 10.3 µmol/L.

20 Controls included patients who had arterial thrombosis (n = 31) or venous thrombosis (n = 30) during follow-up.
distribution, compared with levels below the 90th percentile, were associated with a statistically nonsignificant 70% increased risk of MI (RR, 1.7; 95% CI, 0.9-3.3) after adjustment for other CV risk factors.58 These results differed somewhat from the results of an earlier analysis in this same population (based on 5 years of follow-up and including 271 cases of MI and their matched controls)76 that indicated a statistically significant 3-fold increased risk of MI (RR, 3.4; 95% CI, 1.3-8.8) for elevated homocysteine. Together these results suggest that the strength of the homocysteine-CVD association may decrease

<table>
<thead>
<tr>
<th>Elevated Homocysteine</th>
<th>RR (95% CI)</th>
<th>Adjusted Factors</th>
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<tr>
<td><strong>Studies</strong> Basal tHcy &gt;14.4 µmol/L (75th percentile for controls)</td>
<td>2.0³ (1.4-2.9)</td>
<td>Age, sex, total/HDL cholesterol ratio, smoking, systolic blood pressure</td>
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<tr>
<td><strong>Studies</strong> Basal tHcy &gt;16.6 µmol/L (95th percentile for controls)</td>
<td>0.8⁴ (0.3-2.4)</td>
<td>Age, smoking, diabetes, high blood pressure, Quetelet index, aspirin assignment, total/HDL cholesterol ratio, fasting time, follow-up time</td>
</tr>
<tr>
<td><strong>Studies</strong> Basal tHcy &gt;15.4 µmol/L</td>
<td>4.7⁴ (1.1-20.0)</td>
<td>Age, town, social class, BMI, hypertension, history of diabetes, smoking, alcohol consumption, HDL cholesterol, packed cell volume, serum creatinine concentration, forced expiratory volume</td>
</tr>
<tr>
<td><strong>Studies</strong> Peak PML Hcy² ≥24.0 µmol/L</td>
<td>40.3³ (2.3-710.1)</td>
<td>. . .</td>
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<tr>
<td><strong>Studies</strong> Basal tHcy &gt;17.1 µmol/L (90th percentile for controls)</td>
<td>5.7 (1.8-17.4)</td>
<td>Age, sex</td>
</tr>
<tr>
<td><strong>Studies</strong> Basal tHcy &gt;15.4 µmol/L</td>
<td>4.7 (1.1-20.0)</td>
<td>Age, sex, total/HDL cholesterol ratio, smoking, systolic blood pressure</td>
</tr>
<tr>
<td><strong>Studies</strong> Peak PML Hcy² &gt; mean + 2 SDs for sex- and menopause-matched controls</td>
<td>32.8³ (1.8-605.9)</td>
<td>. . .</td>
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<tr>
<td><strong>Studies</strong> Fasting tHcy &gt; mean + 2 SDs for sex- and menopause-matched controls</td>
<td>4.4³ (0.7-28.9)</td>
<td>. . .</td>
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<tr>
<td><strong>Studies</strong> PML tHcy &gt; mean + 2 SDs for sex- and menopause-matched controls</td>
<td>12.9³ (1.3-124.7)</td>
<td>. . .</td>
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</tbody>
</table>
over time. Indeed, further analyses stratifying by the time since randomization (3.75 years) indicated that the effect of elevated tHcy level was limited largely to the earlier follow-up period (≥3.75 years: RR, 2.8; 95% CI, 0.9-8.4; >3.75 years: RR, 1.3; 95% CI, 0.5-3.1).58

The relationship of plasma homocysteine level with risk of angina pectoris with subsequent coronary artery bypass surgery was also examined in the Physicians’ Health Study.70 During 9 years of follow-up, 149 participants reported newly diagnosed angina pectoris and had subsequent coronary artery bypass surgery. One control subject was matched to each case (age, smoking, length of follow-up). There was no difference in mean homocysteine level (10.9 vs 10.4 µmol/L) or in the proportion with elevated homocysteine levels (7% of cases and controls had homocysteine levels >15.8 µmol/L) between cases and controls. The multivariate RR of angina pectoris with subsequent coronary artery bypass surgery was 1.1 (95% CI, 0.4-3.2).

Recent data from the Multiple Risk Factor Intervention Trial indicated no relationship between baseline homocysteine level and subsequent risk of nonfatal MI or CHD death.60 Cases were 93 nonfatal MIs (occurring within 7 years of sample collection) and 147 CHD deaths (most occurring more than 11 years after sample collection), with controls matched (2:1) to the cases by age, smoking status, clinic, race, and study group (special intervention or usual care). Similar mean levels of homocysteine were observed for MI cases and controls (12.6 vs 13.1 µmol/L) and for CHD deaths and controls (12.8 vs 12.7 µmol/L). The RR for the combined end point of CHD deaths and MI, comparing the highest and lowest quintiles of homocysteine, was 0.9 (95% CI, 0.6-1.6).

A prospective cohort study of 587 patients in Norway with angiography-documented CHD indicated a graded relationship between plasma homocysteine concentrations and subsequent mortality.61 Analysis was based on 64 deaths (50 deaths from CV causes) observed during a median follow-up of 4.6 years. Compared with patients with baseline homocysteine levels below 9 µmol/L, mortality ratios for increasing homocysteine categories were 1.9 (9.0-14.9 µmol/L), 2.8 (15.0-19.9 µmol/L), and 4.3 (≥20.0 µmol/L).

In the British United Provident Association study of 21520 men followed up for an average of 8.7 years, mean homocysteine level was significantly higher in 229 men who died of ischemic heart disease (and who were without a history of ischemic heart disease at baseline) than in 1126 age-matched controls (13.1 vs 11.8 µmol/L).59 Multivariate RR for ischemic heart disease mortality, comparing the highest and lowest quartiles of homocysteine, was 2.9 (95% CI, 2.0-4.1).

Finally, prospective data from the Atherosclerosis Risk in Communities Study indicate no association of homocysteine and subsequent risk of CHD.62 Baseline homocysteine levels for 232 incident cases of CHD (identified during an average follow-up of 3.3 years) were similar to levels for a reference cohort sample of 537 participants (geometric mean, 8.9 vs 8.5 µmol/L). The RR of CHD, comparing the highest and lowest quintiles of homocysteine, was 1.3 (95% CI, 0.5-3.2).

Cerebrovascular Disease. There have been 4 prospective studies of homocysteine and cerebrovascular disease, including 3 nested case-control studies and 1 prospective cohort study composed of patients with systemic lupus erythematosus (SLE) (Table 2 and Figure 2).

In the Finnish study, there was no difference in mean homocysteine level in a comparison of 74 stroke cases and 269 controls matched for sex and age.56 The RR associated with high homocysteine level in this population [RR, 1.3; 95% CI, 0.6-2.4] was based on an analysis of a combined end point of MI [n = 191] or stroke [n = 74] and is displayed only in Table 1 and Figure 1.

In the Physicians’ Health Study, baseline blood levels of homocysteine for 109 participants who developed ischemic stroke during 5 years of follow-up were compared with those of 427 controls matched for age, smoking status, and length of follow-up.73 There was no significant difference in mean homocysteine level at baseline (cases, 11.1 µmol/L; controls, 10.6 µmol/L) or in the proportion of cases and controls whose plasma homocysteine levels exceeded the 95th percentile of the control distribution. Multivariate RR of ischemic stroke, comparing homocysteine levels above the 95th percentile of the control distribution with levels below the 90th percentile, was 0.8 (95% CI, 0.3-2.4).

In the British Regional Heart Study of 5665 men aged 40 to 59 years, baseline plasma homocysteine concentrations for 107 cases who suffered a first stroke during 12.8 years of follow-up were significantly higher than the baseline level for 134 controls (geometric mean: cases, 13.7 µmol/L; controls, 11.9 µmol/L; P < .01) frequency matched by town and age group.74 Comparison of homocysteine levels of 15.4 µmol/L or more with levels less than 10.3 µmol/L indicated a multivariate RR of stroke of 4.7 (95% CI, 1.1-20.0). Men who developed stroke also reported more CHD (37.4% vs 5.1%) and hypertension (67.3% vs 23.7%) at baseline than did controls.

In a prospective cohort study of 337 patients with SLE followed up for an average of approximately 5 years, 29 patients developed stroke.75 Mean plasma homocysteine level at baseline was higher in patients who developed stroke than in patients who did not (10.3 vs 7.4 µmol/L; P < .01). Elevated levels of homocysteine were not defined. However, in multivariate analysis, a change of 1 unit in log-transformed plasma homocysteine concentration was associated with an RR for stroke of 2.4 (95% CI, 1.0-5.8).

EVALUATION OF EPIDEMIOLOGICAL EVIDENCE

Results from cross-sectional and case-control studies generally support an association of elevated homocysteine levels with increased risks of CVD. Results from prospective studies, however, tend to indicate a weak or no association. In evaluating the totality of evidence, it is important to consider the relative strengths and limitations of the
various study designs, including how the temporal sequence of sample collection and disease occurrence may have impacted the study results.

In case-control studies, differences in the recall or ascertainment of exposure history according to disease status are always an important potential source of bias. In blood-based case-control studies, there is an additional concern that the disease process itself may alter blood levels of possible determinants of disease. For example, several investigators have shown that homocysteine concentrations vary with time in patients with stroke or MI and that the time at which blood samples are collected relative to the acute CV event needs to be considered in interpreting results. Lindgren et al reported similar levels of homocysteine in stroke patients and in controls when blood samples were collected in the acute phase of stroke (mean, 2 days after stroke) but observed higher homocysteine levels for cases when blood was collected during the convalescent phase of stroke. Plasma homocysteine concentrations in control subjects did not change over time. Similar increases in plasma homocysteine level during a period of 6 to 8 weeks after acute MI have also been reported.50,53,78 These findings for stroke and MI raise the possibility that the elevated homocysteine concentrations observed in many case-control studies may be a consequence, rather than a possible cause, of the acute CV event. Changes in diet or impaired renal function after MI or stroke may, at least partially, account for elevated homocysteine concentrations. Homocysteine levels have been shown to rise with decreasing renal function and to parallel increases in serum creatinine level.79,80 However, the observation that concentrations of vitamin B6, vitamin B12, and folate, as well as creatinine, do not appear to change markedly after stroke or MI is not consistent with these possibilities. Medications given after the MI (or stroke) may also alter homocysteine levels.81,82 Alternatively, a transient decrease in plasma homocysteine levels after stroke or MI (perhaps as a result of decrease in plasma albumin level) may explain the apparent increase in homocysteine concentration over time. Prospective studies in which homocysteine concentrations are measured before and after MI or stroke may differ in their findings for cases when blood was collected relative to the acute CV event. For example, although higher homocysteine levels were observed in persons with more extensive atherosclerotic disease in a cross-sectional analysis of asymptomatic patients, it is not possible to determine from these data whether the elevated homocysteine levels preceded atherosclerosis, in which case they may be causally related to the disease process, or whether they were secondary to a more immediate cause of atherosclerosis, such as low-grade chronic vascular inflammation.47,60,86 In the latter case, homocysteine level would not be a risk factor for atherosclerotic disease progression, but it might be an indicator of disease severity.

Prospective studies have the major advantage of collecting blood specimens before any relevant clinical events have occurred. Thus, the temporal relationship between elevated homocysteine levels and CVD risk is more clearly defined in prospective studies than in cross-sectional or case-control studies. In addition, information on potential confounders can be collected before the occurrence of relevant clinical events in prospective studies. High homocysteine levels are associated with other predictors of CVD, such as high blood pressure, elevated cholesterol level, and cigarette smoking, and these factors must be accounted for before it is concluded that elevated homocysteine level is an important independent contributor to CVD. As shown in Figures 1 and 2, cross-sectional and case-control studies that included adjustment for potential confounders, either through matching or in analyses, tended to report lower risks of CVD for high homocysteine levels than did studies not adjusting for these factors. However, even with adjustment there is a remaining concern in cross-sectional and case-control studies that the disease process itself may have altered the levels of CV risk factors such as blood pressure and serum cholesterol levels, thereby masking much of the confounding effects of these variables.60 In prospective studies, information on confounders is collected at the beginning of follow-up, thus decreasing any distorting effect the disease process may have on these variables and enabling better control of the confounding effects of these factors.

On the other hand, because the blood samples are stored for extended periods, they may deteriorate over time, accounting for some of the null findings in prospective studies. Most of the available data, however, suggest that blood samples do not deteriorate markedly during storage. For example, blood levels of folate, vitamin B6, and homocysteine from baseline samples stored at −80°C in the Physicians’ Health Study were found to be generally similar to those from fresh plasma collected several years later in follow-up.56,70 Other data also support the stability of plasma homocysteine concentration in stored samples over time, even for samples stored at −20°C for up to 10 years.56,80 However, changes in homocysteine concentration during follow-up, perhaps as a result of changes in diet or medication, could also attenuate the relationship between baseline homocysteine level and risks of CVD in prospective studies. In the Physicians’ Health Study, the 3-fold increased risk of MI for men in the top 5% of baseline homocysteine observed after 5 years was markedly attenuated after 7.5 years of follow-up. Possible one possible interpretation of these findings is that a single baseline measure of plasma homocysteine is a short-term predictor of CVD, but a weak predictor over an extended period, perhaps because of changes in plasma homocysteine concentration over time. Alternatively, the positive findings during
the early years of follow-up in the Physicians’ Health Study may simply have been due to chance.

Finally, although the results of most prospective studies indicate little, if any, relationship between homocysteine levels and risks of CVD, 5 prospective studies have reported subsequent increased risks of CVD for those with high homocysteine levels. Importantly, however, 4 of the 5 studies included patients with preexisting vascular disease. Thus, the link between homocysteine and CVD in these studies is complicated by the probable interrelationships of baseline homocysteine level, prevalent disease, and risk of future events. In the British Regional Heart Study, those who developed stroke also had more CHD (37.4% vs 5.1%) and hypertension (67.3% vs 23.7%) at baseline than did controls. Moreover, homocysteine levels were much higher in controls with CHD at baseline than in controls without CHD (14.3 vs 11.8 μmol/L). Excluding patients with baseline CHD greatly attenuated the association of elevated homocysteine and stroke (data not provided). In the Tromso study, 11.5% of the CHD cases also reported a history of angina pectoris (67.3% vs 23.7%) at baseline than did controls.74 Moreover, homocysteine levels were much higher in controls with CHD at baseline than in controls without CHD (14.3 vs 11.8 μmol/L). Excluding patients with baseline CHD greatly attenuated the association of elevated homocysteine and stroke (data not provided). In the Tromso study, 11.5% of the CHD cases also reported a history of angina pectoris (67.3% vs 23.7%) at baseline than did controls. Moreover, homocysteine levels were much higher in controls with CHD at baseline than in controls without CHD (14.3 vs 11.8 μmol/L). Excluding patients with baseline CHD greatly attenuated the association of elevated homocysteine and stroke (data not provided).

As stated in recent editorials,87,88 many questions remain regarding the relationship of folate, vitamin B$_6$, and vitamin B$_9$ to levels of homocysteine; the relationship of homocysteine to CV risk; and the best ways to demonstrate and recommend risk reduction for individual patients and for populations. The recent findings from prospective studies indicating little predictive ability of plasma homocysteine in CVD underscore the need for a more comprehensive and quantitative overview of all available data. Fortunately, a worldwide overview of observational data is now ongoing, which will provide the most reliable estimates of the strength of the relationship between plasma homocysteine levels and vascular disease (Robert Clarke, MD, written communication, October 29, 1997). Regarding the benefits of supplementation of folate acid, intervention studies have clearly demonstrated that folate acid supplementation, with or without additional supplementation with pyridoxine and cyanocobalamin, reduces plasma levels of homocysteine in those with high levels.43,50,67,89-96 and in healthy subjects.97,101 This has contributed to the enthusiasm for screening for elevated homocysteine. However, randomized trial data regarding a possible benefit of folate supplementation on CVD are nonexistent but urgently needed. Fortunately, the Women’s Antioxidant Cardiovascular Study102 is testing a combination of folic acid (2.5 mg daily), pyridoxine hydrochloride (50 mg daily), and cyanocobalamin (1 mg daily) among 8171 female health professionals, aged 40 years or older, who are at high risk for CVD morbidity and mortality. Results from this and other trials of folate supplementation will provide importantly relevant information on which to base public health recommendations for reducing risks of CVD. Whether these trials will also be able to resolve whether the anticipated decreases in homocysteine are causally related to reduced risks of CVD, thereby supporting the need for screening for elevated homocysteine level, or are merely a marker of less severe disease or improved vitamin status, is less certain. Finally, if shown to be beneficial in reducing risks of CVD, folate supplementation must be an adjunct to, and not a replacement for, proscription of harmful lifestyles. Dietary enrichment with any single factor, despite its appeal and simplicity, cannot be an alternative to the somewhat more demanding, but proven, methods of reducing risks of CVD, such as avoiding cigarette smoking, lowering cholesterol levels, and controlling high blood pressure.

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