Homocysteine and Ischemic Heart Disease

Results of a Prospective Study With Implications Regarding Prevention

Nicholas J. Wald, DSc (Med), FRCP; Hilary C. Watt, MSc; Malcolm R. Law, FRCP; Donald G. Weir, FRCP; Joseph McPartlin, PhD; John M. Scott, ScD

Background: Results from prospective studies of serum homocysteine levels and ischemic heart disease (IHD) are inconclusive. We carried out a further prospective study to help clarify the position.

Methods: In the British United Provident Association (BUPA) prospective study of 21,520 men aged 35 to 64 years, we measured homocysteine levels in stored serum samples and analyzed data from 229 men without a history of IHD at study entry who subsequently died of IHD and 1126 age-matched control subjects (nested case-control design).

Results: Serum homocysteine levels were significantly higher in men who died of IHD than in men who did not (mean, 13.1 vs 11.8 µmol/L; P < 0.001). The risk of IHD among men in the highest quartile of serum homocysteine levels was 3.7 times (or 2.9 times after adjusting for other risk factors) the risk among men in the lowest quartile (95% confidence interval [CI], 1.8-4.7). There was a continuous dose-response relationship, with risk increasing by 41% (95% CI, 20%-65%) for each 5-µmol/L increase in the serum homocysteine level. After adjustment for apolipoprotein B levels and blood pressure, this estimate was 33% (95% CI, 22%-59%). In a meta-analysis of the retrospective studies of homocysteine level and myocardial infarction, the age-adjusted association was stronger: an 84% (95% CI, 52%-123%) increase in risk for a 5-µmol/L increase in the homocysteine level, possibly because the participants were younger; the relationship between serum homocysteine level and IHD seems to be stronger in younger persons than in older persons.

Conclusions: Our positive results help resolve the uncertainty that resulted from previous prospective studies. The epidemiological, genetic, and animal evidence together indicate that the association between serum homocysteine level and IHD is likely to be causal. A general increase in consumption of the vitamin folic acid (which reduces serum homocysteine levels) would, therefore, be expected to reduce mortality from IHD.

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The link between high levels of serum homocysteine and atherosclerotic disease has been suspected since 1969, but several unresolved questions remain. Retrospective case-control studies show a clear relationship with ischemic heart disease (IHD) and there is an association across countries, but prospective cohort studies, which in general provide the most rigorous evidence, have yielded inconclusive results. Studies from Finland and America showed no association between homocysteine level and IHD. The US Physicians’ Health Study reported an association that was limited to persons in the top 5% of the homocysteine distribution (the relative risk compared with those in the lowest 90% was 3.1). In 2 of these studies, there was also no association with stroke and (in one) no association with the development of angina. Results of a fourth prospective study, from Norway, showed a positive association between homocysteine levels and IHD, as did cohort studies of patients with peripheral arterial disease and a prospective study of mortality in patients with coronary artery disease. Other prospective studies showed associations with stroke and arterial thrombotic events. Therefore, an inconsistency exists among the prospective study results: some are negative, whereas others support the association with IHD, stroke, and other thrombotic events demonstrated in retrospective studies.

To help clarify the position, we examined the relationship between homocysteine level and IHD in the prospective British United Provident Association (BUPA) study. We collated results from all the prospective studies and the retrospec-
SUBJECTS AND METHODS

Our prospective study consists of 21,520 men aged 35 to 64 years who were seen at the BUPA medical center in London, England, for a routine medical examination between 1975 and 1982. The study has been described previously. Serum samples were stored at −40°C. We were notified of all subsequent deaths with the assistance of the Office of National Statistics (formerly Office of Population Censuses and Surveys), London. This analysis is based on the 229 men who died of IHD (International Classification of Diseases, Ninth Revision, codes 410-414) by the end of 1987 and who had no history of IHD (angina pectoris or myocardial infarction) on study entry (cases). The mean length of follow-up was 8.7 years. For each case, 5 control subjects (who did not die of IHD and who did not have a history of IHD on study entry) were selected; they were matched for age and duration of storage of the serum sample, both to 1 year. The serum cholesterol level, apolipoprotein A-I and B levels, smoking history, blood pressure, weight, and height of cases and controls were known. Serum samples from these men were retrieved and assayed for homocysteine concentration using the sampling and extraction methods of Araki and Sako and quantitative estimation by high-performance liquid chromatography according to the method of Ubbink et al. Measurements were performed without knowledge of whether the samples were from cases or controls. There were 8 cases and 25 controls with technically unsatisfactory results; the 8 cases were retested with their 40 controls (which included 6 of the 25 controls with unsatisfactory results), so data on all the cases were available. The statistical analysis was based on 229 cases and 1126 controls. Odds ratio estimates were calculated using a conditional logistic regression model. Median homocysteine levels in the controls increased 0.75 µmol/L (95% confidence interval [CI], 0.45-1.06 µmol/L) per 10 years of age; the matching of cases and controls by age allowed for this. Using multiple regression analysis, the odds ratio was adjusted for the weak associations of homocysteine with systolic blood pressure (r = 0.11, P < .001) and serum apolipoprotein B (r = 0.09, P = .003). The odds ratios were not adjusted for serum apolipoprotein A-I level, smoking, or duration of storage of the serum sample because none of these were significantly associated with serum homocysteine level.

We compared our results with those of other epidemiological studies of homocysteine levels and cardiovascular disease, identifying studies from the review by Boushey and colleagues and (subsequent to this) from MEDLINE. Four retrospective studies were omitted because an age-adjusted odds ratio for a specified homocysteine difference was not given or could not be derived from the published results, and studies that measured homocysteine level only after methionine loading were not included. We combined the estimates of the average increase in the risk of IHD with increasing homocysteine levels from different studies (in which an estimated odds ratio was reported or could be calculated from the published data) using the method of Dersimonian and Laird. We also analyzed the odds ratios of risk at different homocysteine levels to determine whether the relationship between homocysteine level and risk of IHD (adjusted) is given by the following equation:

\[ \text{Odds of IHD Death} = \exp(0.0576 \times \text{Increase in Serum Homocysteine Concentration [in micromoles per liter]}) \]

So, for example, a 5-µmol/L increase in serum homocysteine levels increases risk by \( \exp(0.0576 \times 5) \) or 1.33.

RESULTS

Table 1 shows the distribution of IHD risk factors in men who later died of IHD (cases) and men who did not (controls) in the BUPA prospective study; risk factors other than homocysteine level have been reported previously. The mean serum homocysteine concentration was higher in cases than in controls (13.1 and 11.8 µmol/L, respectively; \( P < .001 \)).

Table 2 shows the distribution of homocysteine values in cases and controls; the association with IHD was present across the entire range of homocysteine levels. Table 3 shows the estimated odds ratio of death from IHD according to quartile group of homocysteine level. Among men in the top quartile group, IHD mortality was an estimated 3.7 times that among men in the bottom quartile group, or 2.9 times after adjustment for serum apolipoprotein B levels and systolic blood pressure.

From the continuous logistic regression analysis, an increase in homocysteine concentration of 5 µmol/L (the difference previously used to quantify the dose-response effect) was associated with an increase in the risk of IHD of 41% (odds ratio, 1.41; 95% CI, 1.20-1.65; \( P = .001 \)) before and 33% (odds ratio, 1.33; 95% CI, 1.22-1.59) after adjustment for serum apolipoprotein B level and blood pressure. The dose-response relationship between serum homocysteine level and risk of IHD (adjusted) is given by the following equation:

\[ \text{Odds of IHD Death} = \exp(0.0576 \times \text{Increase in Serum Homocysteine Concentration [in micromoles per liter]}) \]

EPIDEMIOLOGICAL STUDIES

Our data yield 2 main results: (1) a prospective association exists between homocysteine level and risk of IHD and (2) the dose-response relationship is continuous.

Our results help resolve the uncertainty from the previous prospective studies of major IHD events insofar as they corroborate the results of the Norwegian study and the study of patients with peripheral arterial disease and are consistent with the US Physician’s Study. Table 4 shows a summary of the 5 prospective studies of homocysteine level and death from IHD or nonfatal coronary disease.

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myocardial infarction among persons without clinical disease at study entry. The reason for the apparent variation in results, with some studies negative and others positive, is unknown, but the heterogeneity between study results (P = .002) is too large for the overall average to be taken; this would be statistically inappropriate. It is likely that one set is correct and the other is incorrect.

We judge that the positive results are correct for 3 reasons. First, these results are supported by the genetic and animal evidence, as discussed below. Second, measurement error could mask a positive result but could not create one (with case and control samples assayed in the same batches and not distinguished). Third, the measurement error could mask a positive result but could not create one (with case and control samples assayed in the same batches and not distinguished).

**Table 5.** Distribution of Serum Homocysteine Levels in Men Who Died of IHD (229 Cases) and Men Who Did Not (1126 Controls)

<table>
<thead>
<tr>
<th>Quartile Group of Homocysteine, µmol/L</th>
<th>Mean Homocysteine, µmol/L</th>
<th>Odds Ratio (95% Confidence Interval)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10.25</td>
<td>8.77</td>
<td>1.00 (0.74-1.35)</td>
</tr>
<tr>
<td>10.25-12.32</td>
<td>11.26</td>
<td>1.72 (1.29-2.27)</td>
</tr>
<tr>
<td>12.33-15.16</td>
<td>13.56</td>
<td>1.95 (1.47-2.60)</td>
</tr>
<tr>
<td>≥15.17</td>
<td>19.13</td>
<td>3.69 (2.68-5.08)</td>
</tr>
</tbody>
</table>

*Derived from floating absolute risks using the method of Easton et al.20

**Table 6.** Odds Ratio of Death From Ischemic Heart Disease According to Homocysteine Quartile Group, Unadjusted and Adjusted for Apolipoprotein B and Systolic Blood Pressure (Matched Design Allowed for Age)

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**Table 7.** Serum Homocysteine Level and Other Ischemic Heart Disease (IHD) Risk Factors in Men Who Died of IHD (Cases) and Men Who Did Not (Controls)

<table>
<thead>
<tr>
<th>Homocysteine, µmol/L</th>
<th>Cases (n = 229)</th>
<th>Controls (n = 1145)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>6.7 (1.34)</td>
<td>6.2 (1.16)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.22 (0.26)</td>
<td>1.39 (0.33)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.78 (1.61)</td>
<td>1.46 (1.60)</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>38.1 (8.40)</td>
<td>38.9 (8.20)</td>
</tr>
<tr>
<td>Apo B</td>
<td>105 (25.00)</td>
<td>91 (20.00)</td>
</tr>
<tr>
<td>Apo(a)</td>
<td>8.58 (3.49)</td>
<td>5.56 (3.87)</td>
</tr>
<tr>
<td>Weight</td>
<td>79 (10.30)</td>
<td>78 (9.70)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26 (3.10)</td>
<td>25 (2.70)</td>
</tr>
</tbody>
</table>

*Data are given as mean (SD) except for median age at visit and smoking.
†Available on 1126 of the 1145 controls.
‡Available on 62 cases and 237 controls.
§Available on 62 cases and 237 controls.

There are 3 distinct autosomal-recessive inborn errors of metabolism in which homozygotes have very high serum homocysteine levels (about 10-50 times higher than the general population) and very high risk of premature cardiovascular disease: (1) cystathionine β-synthase deficiency, (2) 5,10-methylenetetrahydrofolate reductase deficiency, and (3) the cobalamin metabolic defects that result in impaired methionine synthase activity.37 Heterozygotes for these 3 disorders have serum homocysteine levels about 3 times the population average and high blood pressure.38,39
The only biochemical change in common among these 3 inborn errors of metabolism is a high homocysteine level; no other metabolite is consistently high or low in all 3. Given that cardiovascular disease is also common to all 3 genetic disorders, it follows that it is the homocysteine or a metabolite derived from it that is the cause of the IHD and not that homocysteine is merely a marker of some other cause.

Another genetic defect, affecting about 10% of the population (homozygous for a thermolabile form of 5,10-methylenetetrahydrofolate reductase), also offers a useful natural experiment. This genetic variant leads to moderately raised homocysteine levels (increased by about 50% but with wide variation between studies). In individuals thought to be homozygous on the basis of their phenotype (thermolabile form of 5,10-methylenetetrahydrofolate reductase), the risk of IHD is moderately increased; the combined odds ratio of the 4 cited studies is 3.33 (95% CI, 2.01-5.53). Surprisingly, this is not found in the studies in which cases were defined by the genotype (C677 T mutation); the combined odds ratio for the 6 cited studies is 1.10 (95% CI, 0.71-1.69). The difference between the 2 sets of studies suggests that there may be other, as yet unidentified, genetic defects affecting the enzyme activity, as well as a genetic-environmental interaction causing IHD. In those with the genetic variant, homocysteine levels tend to be more elevated if blood folate levels are low; thus, variation in folate intake may contribute to the variation between studies. Results of these phenotypic studies corroborate the epidemiological evidence in Table 6, showing that the dose-response relationship extends down to so-called normal homocysteine levels.

Results of animal and in vitro experimental studies show that increases in blood homocysteine levels increase the extent of vascular and platelet damage, further
supporting the causal interpretation. Taken together, the epidemiological, genetic, and experimental evidence make a compelling case for a causal relationship between homocysteine and IHD across the range of serum homocysteine levels found in the general population.

**IMPLICATIONS FOR PREVENTION**

Once the relationship between homocysteine level and IHD is judged to be causal, it follows that reducing serum homocysteine levels will reduce the risk of IHD. This can be achieved by increasing consumption of the vitamin folate. A daily folic acid supplement of 0.4 mg/d has been shown by Ward and colleagues to reduce average homocysteine levels in middle-aged patients by 1.9 µmol/L. Assuming this to be accurate, our result (adjusted for other IHD risk factors) indicates that it is equivalent to a 10% reduction in IHD mortality. The true reduction would be somewhat greater than 10% because of the effect of regression dilution bias (the dilution of the effect of a risk factor when based on single measurements that fluctuate in an individual over time). The bias could be allowed for using data from a study recording homocysteine measurements on 2 occasions in the same individuals. There is a need to confirm the size of the effect of folic acid supplementation on serum homocysteine levels and to determine whether there is a dose of folic acid that confers a maximal effect or a homocysteine threshold below which folic acid ceases to reduce serum homocysteine concentration further; this can be accomplished by a relatively small and short-term randomized study of folic acid supplementation.

The existing data together with data from the 2 additional studies proposed would provide the information needed to refine the estimate of the size of the effect of folic acid in the prevention of death from IHD. At present, the magnitude of the benefit remains uncertain, but the evidence shows that an increase in folic acid intake among the general population will lead to a worthwhile reduction in mortality from IHD.

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