Homocysteine and ischaemic stroke in men: the Caerphilly study

U B Fallon, P Elwood, Y Ben-Shlomo, J B Ubbink, R Greenwood, G Davey Smith

Abstract

Objective—To assess the risk of ischaemic stroke associated with total serum homocyst(e)ine (tHcy) concentration.

Design—Cohort study.

Setting—Caerphilly, South Wales.

Participants—2254 men age 50 to 64 years recruited between 1984 and 1988.

Results—107 men developed ischaemic stroke and mean follow up time was 10·2 years. There was no significant difference in mean serum total homocyst(e)ine levels between stroke cases (12·2 µmol 95% CI 11·6 to 13·1) and non-cases (11·7 µmol 95% CI 11·5 to 11·9) (p=0·14). There was no significant risk for a standard deviation increase in homocyst(e)ine (adjusted hazard ratio = 1·1, 95% CI 0·9 to 1·4). An interaction was observed between homocyst(e)ine and age at entry (p=0·003). The adjusted odds ratio comparing the top quintile of homocyst(e)ine with the rest was 2·5 (95% CI 1·0 to 6·2) for strokes occurring under 65 years and 0·5 (95% CI 0·2 to 1·3) at 65 years or older (p value for interaction =0·02). Risk also differed by blood pressure status. The adjusted hazard ratio for a standard deviation increase in homocyst(e)ine was 0·8, (95% CI 0·6 to 1·2) for normotensive men and 1·3 (95% CI 1·1 to 1·7) for hypertensive men (p value for interaction =0·01).

Conclusions—Overall, there is no significant relation between homocyst(e)ine and ischaemic stroke in this cohort. However, its aetiological importance may be greater for premature ischaemic strokes (<65 years) and in hypertensive men.

Increased circulating total homocyst(e)ine (tHcy) concentration in the normal population occurs as a result of either minor genetic abnormalities or nutritional deficiencies of B vitamins such as folic acid. Recently, several studies have been published on the relation between increased serum tHcy concentration and atherosclerotic vascular disease and claims have been made that it is a strong independent risk factor.1 This observation is important because it is biologically plausible2 and because hyperhomocysteinaemia can be easily and cheaply treated with folic acid supplements.3 4 The evidence from cohort studies has been equivocal. Of 11 cohort studies,5 6 7 8 9 10 11 12 13 14 15 six8 9 10 11 12 13 found a positive significant association between increased serum tHcy concentration and CHD, and this disappeared in one of the studies with longer follow up time.14 15 However, there is much less evidence examining the relation between increased serum tHcy concentration and ischaemic stroke. Cross sectional and case-control studies have reported positive findings8 10 11 as have studies that measured carotid artery wall thickness as the primary outcome.12 13 There have been few prospective studies of the association between increased serum tHcy concentration and ischaemic stroke. The British Regional Heart Study25 in a cohort study of all stroke types showed a graded increase per quartile of serum tHcy concentration distribution with a significant trend. Recently the Framingham study has shown similar results with a significant trend across quartiles.26 The Rotterdam study of the elderly found a significant but modest association per 1 mmol change in homocyst(e)ine (odds ratio 1·07, 95% confidence intervals 1·03 to 1·12).17 The US Physicians Study failed to show a significant positive association8 as did a Finnish study.18 Recent systematic reviews have cautioned that there is too much heterogeneity between studies to calculate a quantitative summary estimate in a formal meta-analysis. This is mainly because of the study of diverse populations, little consistency in choice of confounders and use of data derived cut offs.27 28

Methods

DESIGN

The Caerphilly study is a community based prospective study of cardiovascular disease and related outcomes in men age 45 to 59 years who were recruited between 1979 and 1983 from the town of Caerphilly South Wales and the adjacent villages.30 All men in the eligible age group were identified from the electoral register (2818) and invited to participate with an 89% recruitment rate. In 1984, 2398 men aged 50 to 64 years participated in phase II of the study. Serum homocyst(e)ine concentration levels were performed on phase II stored blood samples at a later date. One hundred and forty four men were omitted from the analysis because of prevalent stroke (40) or unavailable serum tHcy concentration level (109) (5 were both). This analysis is based on 2254 men with no prior history of stroke.
WHO criteria but were diagnosed as strokes, and if symptoms or signs suggestive of haemorrhage were not present, they were classified as probable ischaemic strokes. Possible strokes were those in which confirmatory information was lacking but in which it was most probable that the underlying disorder was ischaemic. No attempt was made to distinguish lacunar or other sub-types of stroke from other ischaemic strokes as this is impracticable in epidemiological studies of this nature. Definite haemorrhagic strokes were excluded from the analyses but all other cases were included.

STATISTICAL ANALYSIS
Risk factors for stroke in cases and non-cases were examined using the unpaired t test and z test in the comparison of means and proportions. Total homocyst(e)ine, vitamin B12 and alcohol were not normally distributed and all calculations were done on log transformed data.

Associations between serum tHcy concentration and several potential confounding variables were examined using linear regression. Mean values of serum tHcy concentration were compared in cases and non-cases using the unpaired t test. To compare this analysis with other studies the relation between ischaemic stroke and serum tHcy concentration was examined using several approaches; (a) per unit/standard deviation increase to look for linear effects, (b) trend across quintiles of the tHcy concentration distribution, (c) comparing the top quintile to the remaining 80% and top 5% to the bottom 95% to look for a threshold effect.

Cox proportional hazard modelling was used to perform survival analysis and to adjust for confounders. Date of entry was taken as the date the blood was taken for serum total homocyst(e)ine. Participants were censored at date of ischaemic stroke, date of death or date of end of follow up, which was 31 December 1997.

The risk set was defined using both the dates of entry and age at entry and there was no difference in the results when analysed separately. The proportional hazard assumption for the goodness of fit of the Cox regression model was tested over three intervals of time each containing the same number of stroke events. This was tested for all explanatory variables both graphically using Aalen plots and statistically using the proportional hazard test.

In the final model adjustment is first made for strict confounders, which are age, diabetes, hypertension, systolic blood pressure and current smoking status. The model is then adjusted for factors strongly associated with stroke but not serum tHcy concentration, which are social class and ECG ischaemia. Finally, adjustment is made for factors associated with serum tHcy concentration such as folate, B12, B6, alcohol, body mass index and creatinine.

Interactions between serum tHcy concentration and other variables were explored in the regression model using the likelihood ratio test. Sub-group analyses were performed to assess
the association between stroke and serum tHcy concentration in young men as previous studies have suggested an interaction. Because we wanted to test whether the relation between serum tHcy concentration differed for early (<65 years) and later (≥65 years) stroke, we used a nested case-control study design, so that cases could be defined by age at stroke, in contrast with age at entry for the proportional hazard model. Four controls per case were randomly selected from the comparison group if they were at risk of ischaemic stroke at the same age as the stroke case and using age frequency matching within one year. Logistic regression was used to determine the effect of serum tHcy concentration on stroke in different age strata adjusting for confounders. Interaction was tested using the likelihood ratio test. Subgroup analysis of those with and without hypertension was done using Cox proportional hazard modelling.

Table 2 Serum total homocyst(e)ine and potential confounding variables for ischaemic stroke in the non-cases

<table>
<thead>
<tr>
<th>Linear regression</th>
<th>β coefficient*</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 5 years)</td>
<td>0.04</td>
<td>0.02 to 0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SBP (per 10 mm Hg)</td>
<td>0.006</td>
<td>0.006 to 0.012</td>
<td>0.03</td>
</tr>
<tr>
<td>DBP (per 10 mm Hg)</td>
<td>−0.0002</td>
<td>−0.01 to 0.01</td>
<td>0.97</td>
</tr>
<tr>
<td>Alcohol (per SD in ml/day)</td>
<td>−0.04</td>
<td>−0.05 to −0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body mass index (SD in kg/m²)</td>
<td>−0.11</td>
<td>−0.14 to −0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine (per SD in µmol/l)</td>
<td>0.06</td>
<td>0.04 to 0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol (per SD in mmol/l)</td>
<td>−0.0006</td>
<td>−0.01 to 0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>HDL cholesterol (per SD in mmol/l)</td>
<td>−0.02</td>
<td>−0.03 to −0.007</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides (per SD in mmol/l)</td>
<td>−0.01</td>
<td>−0.02 to 0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Folate (per SD in µg/day)</td>
<td>−0.05</td>
<td>−0.06 to −0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B6 (per SD in µg/day)</td>
<td>−0.04</td>
<td>−0.05 to −0.03</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

‡Mean serum tHcy (µmol/l)

| Never smoked | 11.3 | 1.3 |
| Ex smoker | 11.5 | 1.3 |
| Current smoker | 12.1 | 1.4 |

‡Mean serum tHcy (SD) t test

| Hypertensive | 12.1 | 1.3 |
| Normal tension | 11.6 | 1.3 |
| Manual social class | 11.8 | 1.4 |
| Non-manual social class | 11.5 | 1.3 |
| Diabetic | 10.7 | 1.3 |
| Not diabetic | 11.7 | 1.3 |
| ECG ischaemia | 11.8 | 1.3 |
| Normal ECG | 11.7 | 1.3 |

*Change in homocysteine (natural log) per unit change in risk factor. †Natural log transformed. ‡Geometric mean.
ECG ischaemia, body mass index, creatinine, folate, vitamin B12, vitamin B6 and alcohol.

*Adjusted for age, social class, current smoking, hypertension, systolic blood pressure, diabetes, ECG ischaemia, body mass index, creatinine, folate, vitamin B12, vitamin B6 and alcohol.

The interaction was statistically significant (p=0.02).

There was an interaction between serum $tHcy$ concentration and diastolic blood pressure (p=0.001). The adjusted hazard ratio of ischaemic stroke per standard deviation change in serum $tHcy$ concentration for normotensive men was 0.8 (95% CI 0.6 to 1.2) p=0.3 versus 1.3 (95% CI 1.0 to 1.7) p=0.002 in hypertensive men. The interaction was again significant (p=0.01).

**Discussion**

In this study, a significant association between serum $tHcy$ concentration and ischaemic stroke was not found although weak effect sizes cannot be excluded. The results were consistent when the top 5% and top 20% of the serum $tHcy$ concentration distribution were used as cut off points and when tested for trend per quintile and per standard deviation change in serum $tHcy$ concentration.

The Caerphilly study offers advantages over other studies of the relation between serum $tHcy$ concentration and ischaemic stroke. Survival analysis is used and all data are fully exploited; unlike nested case-control studies all non-cases are part of the comparison group. Ascertainment of stroke status was very detailed and thorough and we have tried to only include ischaemic strokes as cases. We have assumed that those strokes of uncertain type were probably ischaemic. This assumption is reasonable given the age distribution of our events, so that ischaemic strokes would be the predominant type in this population. Initial misdiagnosis of stroke is quite common in clinical settings but is markedly improved by experience, neuroradiology and knowledge of the clinical course. Use of multiple clinical methods is considered sufficient for classifying haemorragic and ischaemic stroke for epidemiological purposes. In addition there was detailed measurement of potential confounders including renal function and dietary factors.

Out of five cohort studies that have been published, three have found a positive association between increased serum $tHcy$ concentration and ischaemic stroke, the British Regional Heart Study, the Rotterdam study of the elderly and recently the Framingham study. None of these studies had dietary measures and none of them included only ischaemic strokes. Our results are consistent with the US Physician’s study where the size and the direction of the effect are very similar but the null hypothesis cannot be rejected because the confidence intervals include one. In contrast with the US Physician’s study, which could be considered a selective well nourished study population, the Caerphilly cohort is composed of predominantly working class men.

If increased serum $tHcy$ concentration is an independent risk factor for cerebrovascular disease as reported in other studies, then why did we not find an effect? Misclassification of exposure because of intraindividual variation of serum $tHcy$ concentration is likely to be random and may have weakened the effect towards the null. A small non-fasting subgroup (3.9%) and possible delay in separating serum may have contributed to this. Instability of stored assays is another possibility but homocyst(e)ine is now thought to be highly stable when stored at −70°C. Misclassification of outcome is another possibility as strokes of uncertain type were classified as ischaemic. However, the association between conventional risk factors and ischaemic stroke was very similar when strokes of uncertain type were included and excluded in separate analysis (data to be published elsewhere). Finally, our null results might be attributable to inadequate power as most previous studies show that any association is of very modest effect.

We observed a relatively high incidence of stroke in our study population. Age standardised mortality rates of stroke in England and Wales are higher than in the USA (MONICA). Age specific stroke death rates for age 45 to 65 years are higher in South Wales than in the England and Wales combined and lowest in the USA (45, 38.9, 32.7 per 100 000 respectively). If the relation between $tHcy$ and stroke is a strong independent causal one as suggested in the literature, then this population based study of predominantly working class men, with a high stroke incidence and reliable measurement of a large number of potential confounding factors is ideally suited to testing this hypothesis.

We performed sub-group analysis for two reasons. Firstly, other papers have reported...
Homocysteine and ischaemic stroke in men

extensively on a differential effect of age and hypertension on serum tHcy concentration. However, more importantly we found a statistically significant interaction between serum tHcy concentration and age and between serum tHcy concentration and diastolic blood pressure. Sixty five years of age was chosen as a cut off because this gave similar numbers of strokes in both groups (50 cases under 65 years and 57 case above). We observed a stronger association between plasma tHcy and rate of ischaemic stroke in men who were under the age of 65 at the time of their stroke in comparison with those who were aged 65 years or over. One of the largest case-control studies of tHcy concentration and stroke was a study of 211 men with premature cerebrovascular disease in young men (mean age 43.7 years). They estimated an odds ratio of stroke of 1.7 (95% CI 1.1 to 2.7) comparing the top quintile of the tHcy concentration distribution with the rest. It is possible that this interaction with age is attributable to chance, though it could well be a real effect. One possible explanation is that tHcy concentration as a risk factor in young men has fewer other risk factors to compete with. As people age, a greater number of risk factors contribute to the overall risk of disease and the apparent risk attributed to tHcy becomes weaker or negligible.

A far greater number of studies have examined the relation between CHD and tHcy. They are generally consistent in suggesting the possibility that there may be a differential age effect of tHcy. Almost all cross sectional and case-control studies of CHD with a positive finding have been of premature CHD with cases < 60 years of age. In two cohort studies, which have found significant associations between tHcy and CHD, the mean age of cases was 53 years and 58 years. The British Regional Heart study is also a cohort of middle aged men.

We found a greater risk of ischaemic stroke associated with tHcy in hypertensive men. The British Regional Heart Study and the Rotterdam study of the elderly found similar results but only the Rotterdam study found a significant interaction. In contrast, the US Physicians reported a greater risk in normotensive men than in those with hypertension as did another study of the elderly.

Total homocyst(e)ine concentration is gaining wide acceptance in the literature as a strong independent risk factor for arterial occlusive disease, including stroke. It is important to recognise that not all cohort studies support this conclusion. A recent systematic review has pointed out that prospective cohort studies are less likely to find a positive result than case-control studies, particularly if they exclude pre-existing vascular disease. Our study supports the hypothesis that any true effect is weak or non-existent and may be only important in certain subgroups. Future randomised controlled trials of folic acid supplementation may provide more robust evidence as to whether interventions that lower tHcy can prevent atherosclerosis and future risk of stroke. In view of our results, such trials would either require very large numbers of participants or recruit subjects at high risk.

Total homocyst(e)ine refers to the sum of the concentrations of free homocysteine, protein-bound homocysteine, the di sulfide homocysteine and the mixed disulfide homocysteine-cysteine.

Conflicts of interest: none.


Homocysteine and ischaemic stroke in men: the Caerphilly study

U B Fallon, P Elwood, Y Ben-Shlomo, J B Ubbink, R Greenwood and G Davey Smith

*J Epidemiol Community Health* 2001 55: 91-96
doi: 10.1136/jech.55.2.91

Updated information and services can be found at:
http://jech.bmj.com/content/55/2/91

These include:

**References**
This article cites 37 articles, 13 of which you can access for free at:
http://jech.bmj.com/content/55/2/91#BIBL

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections

- Cohort studies (794)
- Epidemiologic studies (2838)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/