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 plausible and because hyperhomocysteinaemia can be easily and cheaply treated with folic acid supplements.3 4

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.
The phase II follow up collected the following measurements; medical history, smoking history, London School of Hygiene and Tropical Medicine chest pain questionnaire, height, weight and blood pressure measured with a random zero sphygmomanometer and a 12 lead electrocardiogram (ECG). Detailed methods for these and the wide range of other measures that were made are described elsewhere. For the purposes of this study social class was divided into manual and non-manual. Hypertension was defined as systolic blood pressure over 160 mm Hg, diastolic blood pressure 95 mm Hg and/or self reported hypertension from the questionnaire.

A detailed food frequency questionnaire was self completed before attending the clinic and used to estimate the mean daily dietary intake of nutrients and vitamins. This was validated in a 30% sub-sample with a seven day weighted self completed questionnaire. Participants were asked to return fasting within the next two weeks when a blood sample was obtained. Eighty eight men (3.9%) were not fasting when the blood was taken.

L laboratory Methods

Serum was separated within two hours of obtaining the sample and stored at below −20°C for between 6 and 10 years. Estimations of a wide range of factors relevant to cardiovascular disease were made and described elsewhere. Later, the blood samples were transported on dry ice to South Africa for serum tHcy concentration analysis. Serum total homocyst(e)ine concentration was obtained using a modified method by Araki and Sako of high performance liquid chromatography and was performed in duplicate on every sample. The mean of both measures was used in the analysis. One hundred and twelve samples were split and placed blindly throughout the sample series. The coefficient of variation for these duplicate samples was 9% when one outlier was omitted.

St ink/Outcome Ascertainment

Deaths from stroke comprised all those coded as ICD 430–8 (9th revision of the International Classification of Diseases). Non-fatal strokes were defined clinically according to World Health Organisation criteria as all those cerebrovascular events that produced a neurological deficit that was present for more than 24 hours. Stroke type was then classified by scrutiny of all medical records, including neuroradiology, and where records were poor, the participant or a relative was visited to obtain more information. Summaries of symptoms, signs, past medical history and clinical course were reviewed independently by two clinicians (a stroke specialist and a clinical epidemiologist) and in cases of diagnostic disagreement a third stroke epidemiologist arbitrated. Definite strokes were classified as those that met full WHO criteria and these were further subdivided on the basis of neuroradiology or necropsy findings into infarction or haemorrhage. Probable strokes were those that did not meet full 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.\cite{25, 27} 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 shows the relation between serum tHcy concentration and potential confounding variables in the non-cases. The correlation coefficients correspond to the change in log transformed tHcy concentration with every unit change in risk factor. Established risk factors for ischaemic stroke such as smoking, hypertension, systolic blood pressure and age were positively associated with serum tHcy concentration. Alcohol, folate, vitamin B12, vitamin B6 and body mass index were inversely associated with serum tHcy concentration as was diabetes. There is a significant inverse relation between HDL cholesterol and tHcy concentration but no relation with total cholesterol or triglycerides. Mean serum tHcy concentration was slightly higher in men of manual social class but this was not statistically significant (table 2).

Table 2 shows the risk factors for stroke in the cases and non-cases. Cases were more likely to be older, have hypertension, have a higher mean systolic blood pressure, be diabetic, have evidence of ECG ischaemia, be ever or current smokers and be of manual social class.

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The geometric mean serum tHcy concentration was 12.24 μmol in cases (95% CI 11.6 to 13.1) and 11.7 μmol in non-cases (95% CI 11.5 to 11.9). The difference was not statistically significant (p=0.14). In the Cox proportional hazard model, there was no evidence of departure from the assumption of proportionality. The adjusted hazard ratio of stroke per standard deviation change in serum tHcy concentration was 1.1 (95% CI 0.9 to 1.4) p=0.16. Table 3 illustrates the rate and adjusted rate ratio of stroke comparing each quintile of serum tHcy concentration with the first. There was no significant trend in rate ratio across quintiles of serum tHcy concentration. When the highest quintile of the serum tHcy concentration distribution was compared with the other four quintiles the adjusted hazard ratio for stroke was 1.3 (95% CI 0.8 to 2.0) p=0.3. The adjusted hazard ratio for stroke when the top 5% of the serum tHcy concentration distribution was compared with the rest was 2.0 (95% CI 0.9 to 4.2) p=0.07.

There was a significant interaction between serum tHcy concentration and age in the fully adjusted Cox proportional hazard model (p=0.003) when both were treated as continuous variables. The adjusted odds ratio of stroke when comparing the top quintile of the serum tHcy concentration distribution with the rest, stratified by age at stroke, was 2.5 (95% CI 1.0 to 6.2) p = 0.02 in men who were under 65 years of age, as compared with 0.5 (95% CI 0.2 to 1.2) p=0.16 for men aged 65 years or over.
Table 3  Hazard ratio of stroke comparing each quintile of serum total homocyst(e)ine concentration with the first

<table>
<thead>
<tr>
<th>Quintile of tHcy</th>
<th>n</th>
<th>Cases (n=2112)</th>
<th>Rate 1000 per year</th>
<th>Age adjusted hazard ratio (95% CI) (n=2112)</th>
<th>Hazard ratio adjusted* (95% CI) (n=2112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>455</td>
<td>21</td>
<td>8.2</td>
<td>4.4</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>454</td>
<td>21</td>
<td>10.0</td>
<td>4.5</td>
<td>1.2 (0.6 to 2.2)</td>
</tr>
<tr>
<td>3</td>
<td>444</td>
<td>23</td>
<td>11.4</td>
<td>5.0</td>
<td>1.3 (0.7 to 2.4)</td>
</tr>
<tr>
<td>4</td>
<td>453</td>
<td>14</td>
<td>13.3</td>
<td>3.0</td>
<td>0.8 (0.4 to 1.6)</td>
</tr>
<tr>
<td>5</td>
<td>448</td>
<td>28</td>
<td>19.0</td>
<td>6.4</td>
<td>1.4 (0.7 to 2.6)</td>
</tr>
<tr>
<td>p (trend)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>

*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 sufficiently accurate 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 frozen. 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...
extensively on a differential effect of age and hypertension on serum tHcy concentration.\(^\text{37}^\text{,}^\text{27}\) 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.\(^8\)

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.\(^2\)\(^,\)\(^3\)\(^,\)\(^4\) In two cohort studies, which have found significant associations between tHcy and CHD, the mean age of cases was 53 years\(^3\) and 58 years.\(^4\) The British Regional Heart study is also a cohort of middle aged men.\(^10\)

We found a greater risk of ischaemic stroke associated with tHcy in hypertensive men. The British Regional Heart Study\(^22\) 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\(^23\) as did another study of the elderly.\(^32\)

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.\(^27\) 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 homocysteine refers to the sum of the concentrations of free homocysteine, protein-bound homocysteine, the disulphide homocysteine and the mixed disulphide homocysteine-cysteine. Una Fallon is a Wellcome Trust Training Fellow in Clinical Epidemiology. Conflicts of interest: none.


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