

# Homocysteine and ischaemic heart disease in the Caerphilly cohort

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## Abstract

Elevated circulating total homocyst(e)ine concentrations are associated with a higher prevalence of ischaemic heart disease (IHD). We utilized data from the Caerphilly Prospective Cohort Study to assess the predictive power of the serum total homocyst(e)ine concentration for future IHD. Serum total homocyst(e)ine concentrations were measured in 2290 men in the Caerphilly cohort, a representative population sample of men aged 50–64 years. During a 5-year follow-up period, 56 men suffered fatal IHD, 77 had a non-fatal myocardial infarction, while 21 were found to have ECG evidence of myocardial infarction (MI) when examined at follow-up. The mean serum total homocyst(e)ine concentration in the total of 154 men who experienced an incident IHD event was 12.4  $\mu\text{mol/l}$ , whereas the 2136 men who experienced no such event had a mean level of 11.7  $\mu\text{mol/l}$ . The difference between these means, examined by logistic regression and standardising for the effects of differences in age, social class, smoking, BMI, diabetes, HDL-cholesterol and prevalent IHD is 0.47  $\mu\text{mol/l}$  (95% CI =  $-0.13$  to 1.11  $\mu\text{mol/l}$ ). The mean difference for the 56 men who died, and whose death was attributed to IHD, is 0.81  $\mu\text{mol/l}$  (95% CI =  $-0.17$  to 1.88  $\mu\text{mol/l}$ ) after correction for confounding factors. Vitamin nutritional status and alcohol intake were significant negative determinants of serum total homocyst(e)ine concentrations; the effect of alcohol is explained by the folic acid content of beer, which is the preferred alcoholic beverage in Caerphilly. It is concluded that the serum total homocyst(e)ine concentration is weakly predictive of IHD events, though in the present data adjustments for other factors attenuated the relationship and it became not statistically significant ( $P > 0.05$ ). © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved.

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## 1. Introduction

In the rare inherited disorder homocyst(e)inuria there is impaired clearance of circulating homocyst(e)ine<sup>1</sup> leading to grossly elevated serum and urine levels of homocyst(e)ine [1] and such patients show markedly

accelerated atherosclerosis and thrombosis [2]. Although the evidence is not entirely consistent, lesser degrees of homocyst(e)ine accumulation in the circulation appear to carry an increased risk of death and clinical events consequent upon coronary [3–6], cerebral [7] and peripheral [8] atherosclerosis. Furthermore, positive correlations between the circulating total homocyst(e)ine (tHcy) level and the degree of atherosclerosis judged from coronary angiography have been described [3,9], and mild hyperhomocyst(e)inemia is not infrequently seen in patients with premature vascular disease [10,11]. A meta-analysis of 27 studies has been reported and this has summarised the overall risk in

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<sup>1</sup> Total homocyst(e)ine (tHcy) refers to the sum of free homocysteine, homocystine, homocysteine–cysteine mixed disulphide and protein bound homocysteine concentrations.

terms of an increase in the odds ratio for coronary heart disease of 1.6 for every 5  $\mu\text{mol/l}$  increase in circulating tHcy concentrations in men [12].

A number of mechanisms have been suggested by which elevated tHcy concentrations may relate to cardiovascular disease. These include damage to endothelium cells [13], enhancement of thrombosis [5,11] and stimulation of lipid peroxidation [14,15].

The Caerphilly Prospective Study of cardiac and cerebral ischaemia gives opportunity to further examine the relevance of serum tHcy levels to incident cardiovascular disease in a representative male population sample in the UK. In this report we present data on the predictive power of the serum tHcy concentration for ischaemic heart disease (IHD) during 5 years following the serum tHcy estimations, while evidence on dietary and other 'determinants' of serum tHcy concentrations is also presented.

## 2. Materials and methods

### 2.1. The population sample

The Caerphilly cohort consists of a total sample of men within the defined area of the town of Caerphilly in South Wales and several surrounding villages. A total of 2818 men were identified, and 2512 (89%) co-operated in a population survey in 1979–1983. Five years later, in 1984–88 a further private census was conducted and all the men in the original cohort who were still resident in the area, together with men aged 50–64 years who had moved into the area, were seen again. The present study is based on this 'reconstructed' cohort. It consisted of 2398 men.

### 2.2. Clinic procedure

The men were invited to attend an afternoon or evening clinic. A standard medical history was obtained and the London School of Hygiene and Tropical Medicine (LSHTM) chest pain questionnaire was administered. Smoking history was obtained, height, weight, and blood pressure were measured, and a 12-lead electrocardiogram (ECG) recorded. Detailed methods for these, and for the wide range of other measures which were made, are described elsewhere [16,17].

At the clinic a detailed food-frequency questionnaire [16,18] which the men had been asked to complete with help from their partners before attendance at the clinic, was checked. This was used later, together with food composition tables [19] to give estimates of dietary intakes of certain nutrients and trace elements. Subjects were then asked to return, after an overnight fast, to an early morning clinic where a blood sample was taken between 5 and 10 h. When questioned at the early

morning clinic, all the subjects except 90 (3% of the cohort) confirmed that they were fasting.

### 2.3. Laboratory methods

Serum was separated within 2 h and stored at  $-20$  to  $-70^{\circ}\text{C}$ . Estimations of a wide range of factors of possible relevance to cardiovascular disease were made and are described elsewhere [17]. Samples of serum, which were stored frozen between 6 and 10 years, were later transported on dry ice to the laboratory in Pretoria for estimation of tHcy concentrations. High-performance liquid chromatography analyses were performed in duplicate on every sample using a modification of the method of Araki and Sako [20,21]. This method entails complete reduction of homocystine, the mixed disulphide (cysteine–homocysteine) and release of protein-bound homocysteine. This method therefore measures serum total homocyst(e)ine concentrations (free + protein bound). A total of 112 blind split sample duplicates were mixed in throughout the series. The coefficient of variation for these split sample duplicates was 9.0% if one outlier was omitted.

### 2.4. Follow-up procedure

An attempt was made to identify every IHD event that occurred during the 5 years following the base-line examinations of the men. The surviving men were invited to a clinic at which the LSHTM chest pain questionnaire was asked again, together with questions about hospitalisation for any episode of chest pain and an ECG was recorded. The few men who had moved out of the area were sent a self-administered version of the chest pain questionnaire. Lists maintained for Hospital Activity Analysis (HAA) were also used to identify men who had been admitted to hospitals local to the area with myocardial infarction (MI) (ICD 410–414). The hospital notes and where appropriate, the notes held by the general practitioners were then searched and events that satisfied the World Health Organisation (WHO) criteria for definite MI identified (ICD 410–414). Additional to this, the records of all men in the cohort have been 'flagged' at the UK National Health Service Central Registry and notification of deaths with certified cause is automatic.

Three types of major incident IHD events have therefore been used as described elsewhere [17]. 1) Death due to IHD: all death certificates coded to ICD 410–414. Deaths attributed to other causes have been treated as non-IHD events. 2) Clinical MI: hospitalised events which fulfilled the WHO criteria. 3) ECG MI: events which fulfilled the WHO categories, namely, no Q-QS wave (Minnesota codes 1-1, 1-2, or 1-3) on recruitment and major or moderate Q-QS waves (Minnesota codes in the range 1-1-1 through 1-2-5 plus 1-2-7) on follow-up.

## 2.5. Statistical methods

The distribution of tHcy concentrations was found to be log-normal. All calculations were therefore done on log transformed data, but the means and 95% confidence intervals which are shown in this report have been transformed back into original units. For clarity in presentation of data on relationships, the subjects have been subdivided into approximately equal fifths by the relevant independent variable (e.g. blood pressure, BMI, etc.) The mean serum tHcy concentration has been calculated within these subgroups and standardised for the effects of possible confounding variables including age, social class, BMI, smoking status, diastolic blood pressure, diabetes and serum HDL cholesterol concentration. Subjects with prevalent IHD were not excluded, but the data were standardised as described above for prevalent IHD. Since the prevalence of IHD was carefully documented in our population sample, our approach included standardisation for 'silent' IHD, a factor which is often overlooked in prospective studies. All tests of statistical significance were conducted on the continuously distributed variables, and not on grouped data, so that maximum use was made of the available information.

In order to investigate the relationship between incident IHD and serum tHcy concentration, a logistic regression analysis was performed. The logit of the probability of an IHD event was the dependent variable regressed on serum tHcy concentration and the chosen confounders as independent variables. Serum tHcy concentrations were divided into five groups and entered into the regression as four dummy variables. The exponential of the regression coefficients of these dummy variables gave the approximate relative odds of having an IHD event relative to the groups with the lowest serum tHcy concentrations.

## 3. Results

Serum tHcy concentrations were available for 2290

men. Serum tHcy concentrations of the 90 men who reported that they were non-fasting did not differ significantly from serum tHcy concentrations of the fasting men. This is in agreement with observations tHcy concentrations showed only small variations after breakfast [22] and the non-fasting subjects were therefore retained in the statistical analyses reported below. The frequency distribution for serum tHcy concentrations is skewed to the right, but was gaussian on transformation to logarithms. The mean level, after transformation back to original units, was 11.8  $\mu\text{mol/l}$  and 95% of the observations lay between 7.3 and 24.5  $\mu\text{mol/l}$ .

The serum tHcy concentration is known to be associated with age, and in our data there is a steady and significant monotonic rise from 11.3 (95% CI: 11.0–11.6)  $\mu\text{mol/l}$  in the 495 men aged 50–52 years inclusive, to 12.3 (95% CI: 12.0–12.7)  $\mu\text{mol/l}$  in the 468 men aged 62–64 years inclusive. Linear regression indicates a rise by a factor of 1.07 for 10 years of age within the age range of the cohort. Social class had a trivial but statistically significant association: men in non-manual classes had a mean serum tHcy concentration of 11.6 (95% CI: 11.3–11.8)  $\mu\text{mol/l}$ , and those in manual classes 11.9 (95% CI: 11.7–12.1)  $\mu\text{mol/l}$  ( $P < 0.05$ ). Smoking is also known to be associated with the serum tHcy concentration and in our cohort, the 412 men who had never smoked had a significantly lower serum mean tHcy level of 11.3 (95% CI: 11.0–11.6)  $\mu\text{mol/l}$  compared with 12.1 (95% CI: 11.9–12.3)  $\mu\text{mol/l}$  of the 995 current smokers ( $P < 0.005$ ). Ex-smokers had a mean serum tHcy concentration of 11.6 (95% CI: 11.4–11.9)  $\mu\text{mol/l}$ , which was also significantly lower than the mean serum tHcy concentration in smokers. The possible confounding effects of these observations are allowed for in all that follows.

The mean (S.D.) length of follow up was 61 [6] months and during this period there were 161 WHO defined IHD events for the total cohort ( $n = 2398$ ), 154 in the men ( $n = 2,290$ ) for whom serum tHcy estimations were available. Fifty-six men died and 98 survived

Table 1  
Mean serum total homocyst(e)ine (tHcy) concentrations and 95% confidence intervals in men from the Caerphilly cohort, subdivided by whether or not they experienced an ischaemic heart disease (IHD) event

Parameter	No IHD ( $n = 2136$ )	Non-fatal IHD ( $n = 98$ )	Fatal IHD ( $n = 56$ )	All cases of IHD ( $n = 154$ )
Mean tHcy (95% CI)	11.7 (11.6–11.9)	12.1 (11.3–12.9)	12.9 (11.7–14.2)	12.4 (11.7–13.1)
Adjusted mean tHcy <sup>a</sup> (95% CI)	11.7	12.0 (11.3–12.8)	12.5 (11.5–13.6)	12.2 (11.6–12.8)
Adjusted mean difference from 'no IHD' <sup>a</sup> (95% CI)		0.30 (–0.43–1.06)	0.81 (–0.17–1.88)	0.47 (–0.13–1.11)

Serum tHcy concentrations are expressed in  $\mu\text{mol/l}$ .

<sup>a</sup> Serum tHcy concentrations were adjusted for age, social class, BMI, smoking, diastolic blood pressure, diabetes, HDL cholesterol level and prevalent IHD. Serum total cholesterol concentration did not show a significant association with serum tHcy concentration.

Table 2  
Relative odds and 95% CI for an IHD event in fifths of men in the Caerphilly cohort subdivided by ln tHcy concentration

Median serum tHcy ( $\mu\text{mol/l}$ )	Relative odds for an IHD event		
	A	B	C
Lowest fifth 8.36	1.0	1.0	1.0
Second fifth 10.02	1.1 (0.6–1.9)	1.1 (0.6–2.0)	1.1 (0.6–1.9)
Middle fifth 11.48	1.3 (0.7–2.2)	1.3 (0.7–2.2)	1.2 (0.7–2.1)
Fourth fifth 13.15	1.2 (0.7–2.1)	1.2 (0.7–2.1)	1.1 (0.6–2.0)
Highest fifth 16.74	1.6 (1.0–2.8)	1.5 (0.9–2.6)	1.4 (0.8–2.3)
<i>P</i> for linear trend	0.03	0.06	0.13

95% CIs are reported in brackets.

A: relative odds unstandardised for any confounding variable.

B: relative odds adjusted for age, social class, body mass index, smoking and prevalent IHD.

C: relative odds adjusted for above plus diastolic blood pressure, diabetes and HDL cholesterol level.

an event (77 had a non-fatal clinically identified MI and 21 had ECG evidence alone). Table 1 gives the mean serum tHcy concentrations of those who developed IHD and those who did not, while Table 2 summarises the predictive power of serum tHcy concentrations for IHD. There is a significant gradient in the risk for IHD, but allowing for the effects of confounding variables reduces the gradient and statistical significance is lost.

Table 3 shows that there are weak, but significant associations between serum tHcy concentration and blood pressure (positive), HDL cholesterol and body mass (both negative). There was however no evidence of any significant associations with fasting blood glucose, serum cholesterol or triglyceride concentrations.

Table 3  
Mean serum tHcy concentrations and 95% confidence intervals in fifths of men defined by levels of various risk factors for IHD after adjustment for the effects of age, social class, smoking and prevalent IHD

Fifth of men	Serum tHcy ( $\mu\text{mol/l}$ ) in men divided in fifths according to:					
	Diastolic blood pressure	HDL cholesterol	BMI	Fibrinogen	Plasma viscosity	White cell count
Lowest fifth	11.4	11.9	12.3	12.1	11.6	11.5
Second fifth	11.7 (11.2–12.2)	12.1 (11.7–12.5)	11.8 (11.3–12.3)	11.4(11.0–11.9)	11.8 11.3–12.2)	11.8 11.4–12.3)
Middle fifth	11.8 (11.4–12.3)	11.9 (11.6–12.2)	11.9 (11.4–12.3)	11.6(11.1–12.1)	12.0 (11.5–12.4)	11.5 (11.0–12.0)
Fourth fifth	11.8 (11.3–12.3)	11.6 (11.3–12.0)	11.5 (11.1–12.0)	11.9(11.5–12.4)	11.6 (11.2–12.1)	11.9 (11.5–12.4)
Highest fifth	12.1 (11.6–12.6)	11.3 (11.0–11.6)	11.4 (10.9–11.9)	11.8(11.3–12.3)	11.8 (11.4–12.3)	12.0 (11.5–12.5)
<i>P</i> for linear trend	<0.05	<0.005	<0.0005	ns	ns	<0.0005

CIs are reported in brackets. Diastolic blood pressure has been adjusted for body mass index (BMI), while HDL cholesterol has been adjusted for BMI as well as alcohol consumption. No significant associations were found with serum concentrations of triglycerides and total cholesterol, nor with fasting blood glucose levels.

There were 81 subjects with diabetes and they had a mean serum tHcy concentration of 10.7 (95% CI: 10.0–11.4)  $\mu\text{mol/l}$ , which was significantly lower than the mean of 11.8  $\mu\text{mol/l}$  observed in the rest of the cohort ( $P < 0.005$ ). The extent to which all these factors confound relationships has been allowed for in the examinations of relationships with IHD (Tables 1 and 2).

Table 3 also displays associations with three factors involved in haemostasis: fibrinogen, plasma viscosity and white cell count. Elsewhere it has been shown that these are powerful predictors of IHD [17]. Only white cell count, but neither fibrinogen nor viscosity, shows a significant relationship with serum tHcy concentration.

Possible dietary determinants of serum tHcy concentrations are shown in Table 4. Intakes have been estimated with the use of a food frequency questionnaires completed by each man, as described by Fehily et al. [16]. The dietary elements examined (vitamin B-6, vitamin B-12, folate) are those which have been identified in previous publications as of relevance [23,24]. The nutritional status of these vitamins show strong and significant associations with the serum tHcy concentration in the present data. Dietary folate intake is of particular interest and in our data this is strongly and negatively related to the serum tHcy concentration. We do not have measures of plasma or red cell folate for the men in the cohort, but mean corpuscular volumes (MCV), which are dependent upon an adequate folate and vitamin B-12 intake, were obtained. We therefore investigated the relation between MCV and serum tHcy concentration. Table 5 shows that serum tHcy concentrations rise markedly at high MCVs, the highest 5% of men in the total cohort (109 men) having a mean of 15.1 (95% CI: 14.2–16.1)  $\mu\text{mol/l}$  compared with the overall mean of 11.8  $\mu\text{mol/l}$ . All these 109 men had MCVs > 100 fL (a criterion of possible megaloblastic anaemia), and within them there is a very marked positive correlation between serum tHcy concentration and MCV, the mean serum tHcy concentration rising

Table 4  
Mean serum tHcy concentrations and 95% CIs in fifths of men defined by dietary vitamin intakes, after adjustment for the effects of age, social class, body mass index, smoking, total energy intakes and prevalent IHD

Fifth of men	Serum tHcy concentrations ( $\mu\text{mol/l}$ ) in men divided into fifths according to dietary intake of:		
	Folate	Vitamin B-12	Vitamin B-6
Lowest fifth	13.5 (13.1–13.9)	12.8 (12.4–13.2)	13.2 (12.7–13.6)
Second fifth	12.6 (12.1–13.1)	12.0 (11.5–12.5)	12.0 (11.5–12.5)
Middle fifth	11.6 (11.2–12.1)	11.7 (11.3–12.2)	11.7 (11.3–12.2)
Fourth fifth	11.0 (10.5–11.4)	11.3 (10.8–11.8)	11.3 (10.8–11.8)
Highest fifth	10.4 (9.9–10.9)	11.0 (10.6–11.5)	10.7 (10.3–11.3)
<i>P</i> for linear trend	<0.0005	<0.0005	<0.0005

95% CIs are reported in brackets.

to 19.9  $\mu\text{mol/l}$  in the 1% of men ( $n=20$ ) with the highest of all MCVs.

Serum tHcy concentrations showed a significant negative relationship with alcohol intake (Table 6). Dietary folate intakes showed a significant and strong association with alcohol intake. This is explained by the folate content of beer (the preferred alcoholic beverage in Caerphilly), which is approximately 9  $\mu\text{g}/100$  ml [19]. The relationship between serum tHcy concentrations and alcohol intake is substantially weakened when the data is standardised for folate intakes.

#### 4. Discussion

The data presented here come from a large representative population cohort of older men within which estimations of tHcy were made on almost every member of the cohort. Although the association between serum tHcy concentration and the risk of an incident IHD event is significant, this relation is weakened when

the data is standardised for the usual confounding factors including age, social class, body mass index, smoking and prevalent IHD (Table 2). In addition, our data revealed significant relations between serum tHcy concentrations and diastolic blood pressure, diabetes, and HDL cholesterol level (Table 3). The role of these three factors in IHD have been well documented, and when we standardised for the latter three factors as well, the significance of the association between IHD and serum tHcy concentrations was lost. This suggests that the association between IHD and serum tHcy concentrations may have been mediated by other risk factors. The serum tHcy concentration did not feature as an independent IHD risk factor in the Caerphilly cohort.

The difference in mean serum tHcy concentrations between the 154 men who suffered an IHD event (12.4  $\mu\text{mol/l}$ ) and those who did not (11.7  $\mu\text{mol/l}$ ), is equivalent to +5.9% and is not statistically significant. Our results are similar with those found in the US Physicians Health Study [6], in which 271 men with MI had a mean plasma tHcy concentration of 11.1  $\mu\text{mol/l}$ , compared to 10.5  $\mu\text{mol/l}$  in the same number of paired controls, a difference of 5.7%. In the latter study, which utilised a nested case-control design, the above mentioned difference of 5.7% was statistically significant, which reflects the larger number of myocardial infarction patients in that study. In the Tromsø Health Study [25] the differences between patients and controls were more accentuated; the mean (S.D.) serum tHcy concentration was 12.7 (4.7) in 123 cases with myocardial infarction compared to 11.3 (3.7) in controls, a difference of 11.7%. On the other hand, Alfthan et al. [26] recently published results from their prospective case/control study showing no relation between plasma tHcy concentration and IHD in a Finnish population. In contrast to other IHD prone populations which display a serum tHcy concentration frequency distribution which is skewed to the right [6,25], the frequency distribution approaches normality in the Finnish population [26]. This suggests that aberrant serum homocyst(e)ine

Table 5  
Association between tHcy and mean cell volume (MCV)

Mean cell volume	Mean serum tHcy concentration ( $\mu\text{mol/l}$ )	
	Uncorrected	Standardized for alcohol intake
Lowest fifth	11.7 (11.4–12.0)	11.6 (11.3–11.9)
Second fifth	11.4 (10.9–11.8)	11.3 (10.9–11.7)
Middle fifth	11.7 (11.2–12.2)	11.7 (11.2–12.1)
Fourth fifth	11.6 (11.2–12.1)	11.7 (11.2–12.1)
Highest fifth	12.5 (12.0–13.0)	12.7 (12.2–13.2)
<i>Within highest fifth:</i>		
lowest quarter	11.6 (10.9–12.3)	11.7 (11.0–12.4)
second quarter	11.3 (10.6–12.0)	11.5 (10.8–12.2)
third quarter	12.5 (11.7–13.3)	12.7 (11.9–13.5)
highest quarter	15.1 (14.2–16.1)	15.4 (14.5–16.4)

Mean serum tHcy concentrations and 95% CI's in groups of men defined by mean cell volume.

95% CIs are reported in brackets.

Table 6  
Mean serum tHcy concentrations and dietary folate intakes in men grouped by alcohol intake

Group	Serum tHcy ( $\mu\text{mol/l}$ )	Serum tHcy ( $\mu\text{mol/l}$ ) standardised for folate intake	Mean folate intake ( $\mu\text{g/day}$ )
Abstainers	12.9 (12.2–13.6)	12.5 (11.9–13.2)	233 (59)
Lowest quarter	12.3 (11.6–13.0)	11.9 (11.3–12.6)	248 (60)
Second quarter	11.8 (11.1–12.4)	11.5 (10.9–12.1)	256 (56)
Third quarter	11.5 (10.9–12.1)	11.6 (10.9–12.2)	281 (57)
Highest quarter	11.1 (10.5–11.8)	12.0 (11.3–12.8)	367 (88)
<i>P</i> for linear trend	<0.0005	0.012	<0.0005

Serum tHcy concentrations were adjusted for the effects of age, social class, body mass index, smoking, total energy intakes and prevalent IHD. Values in brackets are 95% CIs (serum tHcy) or standard deviations from the mean (folate intake).

concentrations are rare in this population group and may therefore be expected to contribute little to the development and progression of IHD.

In the meta-analysis of Boushey et al. [12], the predictive power of fasting tHcy was measured in nine studies, and the relationship in men was summarised as an increase in the odds ratio for coronary heart disease of 1.6 for every 5  $\mu\text{mol/l}$  increase in the circulating tHcy concentration. In our data the excess risk per 5  $\mu\text{mol}$  tHcy (untransformed) is only 1.2 (95% CI 1.0–1.3). This difference is partly explained by the fact that Boushey and coworkers' meta-analysis included seven retrospective studies on serum tHcy concentrations and IHD risk. In almost all the retrospective studies, the differences between IHD cases and controls were considerably larger than recently found in prospective studies. This is eloquently described by Ueland et al. [27], who summarized the retrospective studies published before 1992 and found that circulating homocyst(e)ine concentrations were 30% higher in IHD cases compared to controls.

The small difference between men who experienced an IHD event and the other men in this and in at least one other prospective study [26] raises questions about the clinical relevance of higher serum tHcy concentrations. It may be argued that the small differences between patients and controls with respect to serum tHcy concentrations in prospective studies is the result of regression dilution bias [28]. It might further be argued that part of the social class association with IHD is mediated by serum tHcy concentrations, and therefore standardisation for social class represents over-control. This is not so, however, because the omission of social class from the regression summarised in Table 2 makes virtually no change to the data.

The magnitude of the differences in tHcy concentrations between subjects who went on to experience an IHD event and the others, is in fact similar as observed for serum cholesterol concentrations in the prospective studies mentioned above [6,25,26]. This emphasises the fact that small differences do not necessarily invalidate the role of a risk factor in the pathogenesis of IHD. However, it should be pointed out that in contrast to

cholesterol, which role in atherosclerotic plaque formation and progression has been well established, the role of slightly raised serum tHcy concentrations on IHD progression still lacks biological plausibility, which is one of the criteria of causality [29]. It has been suggested that the effects of homocyst(e)ine on endothelial cells [30], LDL peroxidation [14,15], smooth muscle cell proliferation [31], and blood coagulation [2], indicate that elevated serum tHcy concentrations is a plausible IHD risk factor [12]. However, above mentioned homocyst(e)ine effects are only seen at very high concentrations, may often also be induced by cysteine (which shows no relation with IHD), and are therefore unlikely to implicate circulating tHcy levels in the pathogenesis of IHD. More clinical and epidemiological evidence is required to assess the possible relative contribution of elevated serum tHcy concentrations to IHD risk, while an overview of currently available data will be useful to evaluate the status of serum tHcy concentration as IHD risk factor.

Although the clinical relevance of a raised serum tHcy concentration with respect to IHD remains unproven, our data do indicate that an elevated serum tHcy concentration should not be ignored. High serum tHcy concentrations are strongly associated with an increased MCV, reflecting changes in erythrocyte morphology characteristic of a folate and/or vitamin B-12 deficiency [32]. The relationship between tHcy concentration and MCV is explained by the effect of folate status on both parameters. Folate, required in the remethylation of homocysteine to methionine [33], is an important determinant of circulating tHcy concentrations [23,34] and folate supplementation may be effectively used to treat hyperhomocyst(e)inemia [35]. A high serum tHcy concentration reflects inadequate folate and/or vitamin B-12 status which increases the risk for megaloblastic anemia, and it may be prudent to treat hyperhomocyst(e)inemia with appropriate vitamin supplementation.

Alcohol consumption emerged as a strong determinant of serum tHcy level, which makes it a potential confounding factor, being related negatively to both IHD incidence and the serum tHcy level. In the case of

the Caerphilly cohort, the effect of alcohol was mediated through its folate content. The most widely consumed alcoholic beverage in Caerphilly is beer, which contains approximately 9  $\mu\text{g}$  of folate per 100 ml [19]. It is therefore suggested that a relatively high beer intake may modulate serum tHcy concentrations as result of its folic acid content. The association between serum tHcy concentration and alcohol consumption may be weaker or even absent in populations where other types of alcoholic beverages are preferred. Unfortunately, no other author seems to have examined the effect of alcohol closely within their data. Reis et al. [36] excluded alcoholics from their study. In the US Physicians study, Stampfer et al. [6] found a weak correlation between alcohol intake and serum tHcy level, but they did not include alcohol in their model. Clarke et al. [4] stated that they adjusted their odds ratios 'for the usual risk factors', but these do not appear to have included alcohol intake. The effect of alcohol should be examined more closely in other datasets, bearing in mind that to allow for the effect of alcohol might result in over control and the loss of a possible biologically significant association.

In summary, our data weakly support previous observations that the plasma tHcy concentration is predictive of future cardiovascular disease events, but the significance of the association is lost once the effects of other risk factors are allowed for.

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