Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men

John C Chambers, Omar A Obeid, Helga Refsum, Per Ueland, David Hackett, James Hooper, Rebecca M Turner, SImon G Thompson, Jaspal S Kooner

Summary

Background Reasons for the increase in mortality due to coronary heart disease (CHD) in UK Indian Asians are not well understood. In this study, we tested the hypotheses that elevated plasma homocysteine concentrations are a risk factor for CHD in Indian Asians, and explain part of their increased CHD risk, compared with Europeans.

Methods We undertook two parallel case-control studies, one in Europeans and one in Indian Asians. We recruited 551 male cases (294 European, 257 Indian Asian) and 1025 healthy male controls (507 European, 518 Indian Asian). Fasting and post-methionine load homocysteine, vitamin B_{12} and folate concentrations, and conventional CHD risk factors were measured.

Findings Fasting homocysteine concentrations were 8% higher (95% CI 3–14) in cases compared with controls, in both ethnic groups. The odds ratio of CHD for a 5 μmol/L increment in fasting plasma homocysteine was 1·3 (1·1–1·6) in Europeans and 1·2 (1·0–1·4) in Indian Asians. The association between fasting plasma homocysteine and CHD was independent of conventional CHD risk factors in both ethnic groups. Post-load homocysteine concentrations were not significantly different in cases compared with controls. Among the controls, fasting homocysteine concentrations were 6% (2–10) higher in Indian Asians than in Europeans. From the results we estimate that elevated homocysteine may contribute to twice as many CHD deaths in Indian Asians, compared with Europeans. The differences in homocysteine concentrations between the two ethnic groups were explained by lower vitamin B_{12} and folate levels in Asians.

Interpretation Plasma homocysteine is a novel and independent risk factor for CHD in Indian Asians, and may contribute to their increased CHD risk. Raised homocysteine concentrations in Indian Asians may be related to their reduced vitamin B_{12} and folate levels, implying that the increased CHD risk in this group may be reduced by dietary vitamin supplementation.

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See Commentary page xxx

National Heart and Lung Institute (J C Chambers MRCP, J S Kooner FRCP)**, and Department of Medical Statistics and Evaluation** (R M Turner MSc, S G Thompson DSc)**, Imperial College School of Medicine, Hammersmith Hospital, London; Department of Cardiology, Ealing Hospital, Middlesex** (J C Chambers, J S Kooner)**; Department of Human Nutrition, St Bartholomew's and Royal London School of Medicine & Dentistry, London** (O A Obeid PhD)**; Hemel Hempstead General Hospital, Hemel Hempstead** (D Hackett FRCP)**; Department of Clinical Biochemistry, Royal Brompton Hospital, London, UK** (J Hooper MD)**; and Department of Pharmacology, University of Bergen, Norway** (H Refsum MD, P Ueland MD)

Correspondence to: Dr Jaspal S Kooner, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK
(e-mail: j.kooner@ic.ac.uk)

Introduction

Mortality due to coronary heart disease (CHD) is 40% higher in Indian Asians compared with Europeans.[1] Increased CHD mortality in Indian Asians is not accounted for by cigarette smoking, hypercholesterolaemia, or hypertension.[2,3] Diabetes and insulin resistance are more prevalent in Indian Asians than in Europeans,[2–4] and account for a high proportion of their CHD risk.[3,5] However, the full explanation for the increased CHD risk in Indian Asians is unknown.

Homocysteine is a sulphur-containing aminoacid, plasma concentrations of which are determined by genetic factors and nutritional deficiencies of vitamin B_6, B_{12}, and folic acid.[6] Plasma homocysteine is increasingly recognised as an independent risk factor for vascular disease.[6–11] Homocysteine concentrations above the 80th centile of normal have been reported in almost 40% of patients with vascular disease, including CHD.[8] Meta-analysis of 17 studies involving 5230 individuals suggests that a 1 μmol/L increment in homocysteine concentrations is associated with a 10% increase in CHD risk.[12] Most previous studies have been undertaken in North American and European populations, and the importance of plasma homocysteine as a risk factor for CHD in Indian Asians, in whom the CHD risk is greater than in Europeans, is not known.

Indian Asians are reported to have reduced intakes of vitamin B_{12} and folate, the nutritional determinants of homocysteine.[13,14] We therefore tested the hypotheses that high concentrations of plasma homocysteine are a risk factor for CHD in Indian Asians, and explain part of their increased CHD risk, compared with Europeans.

Patients and methods

Patients

Two parallel case-control studies were done, one in Europeans, and one in Indian Asians. Indian Asians were the main focus of the study, but including Europeans enabled direct comparison of contemporary results between the two ethnic groups, and estimation of the extent to which homocysteine may explain the increased CHD risk in Indian Asians.

We investigated 551 male patients with CHD (294 European, 257 Indian Asians), and 1025 healthy male controls (507 European, 518 Indian Asian) between 1995 and 1998. Indian Asians were resident in the UK for a mean of 27 years (SD 8), and had all four grandparents of north Indian descent; Europeans were white, and were born in the UK. Consecutive CHD patients were identified from the cardiology outpatient department, coronary care unit, and coronary angiography records of three west London hospitals. Patients were aged less than 60 years at time of diagnosis, and not studied within 3 months of myocardial infarction or coronary intervention. The acceptance rate for cases was 65%. European and Indian Asian male controls were identified at random from the age-sex register of 56 local general practitioners. We sent invitation letters, by post, to 2400 controls (1200 European, 1200 Indian Asian; age 35–60 years). To estimate how many individuals actually received the invitation, we visited the homes of 250, and found that 55 (22%) were no longer at the stated address We therefore inferred that the letter

	Europeans			Indian Asians			p*
	Cases (n=294)	Controls (n=507)	p	Cases (n=257)	Controls (n=518)	p	
Clinical							
Age (years)	55·3 (5·9)	49·4 (6·5)	<0·001	52·0 (7·3)	49·0 (6·9)	<0·001	>0·1
Body-mass index (kg/m²)	27·6 (3·9)	26·7 (4·0)	0·003	26·8 (3·7)	26·9 (3·5)	>0·1	>0·1
Social class (non-manual)	129 (50%)	272 (56%)	0·08	88 (43%)	187 (39%)	>0·1	<0·001
Systolic blood pressure (mm Hg)	129 (19)	128 (19)	>0·1	129 (20)	131 (21)	>0·1	0·01
Diastolic blood pressure (mm Hg)	81 (10)	81 (11)	>0·1	81 (12)	83 (12)	0·01	0·001
Hypertension	130 (45%)	109 (22%)	<0·001	127 (49%)	191 (37%)	0·001	<0·001
Biochemical							
Glucose (mmol/L)†	5·47 (1·20)	5·26 (0·91)	0·006	6·25 (2·07)	5·67 (1·49)	<0·001	<0·001
Diabetes	29 (10%)	20 (4%)	0·001	92 (36%)	80 (16%)	<0·001	<0·001
Total cholesterol (mmol/L)	5·40 (1·11)	5·64 (1·02)	0·002	5·24 (1·12)	5·63 (1·02)	<0·001	>0·1
HDL cholesterol (mmol/L)	1·20 (0·34)	1·33 (0·38)	<0·001	1·13 (0·29)	1·22 (0·37)	0·001	<0·001
Triglycerides (mmol/L)†	1·60 (0·91)	1·31 (0·76)	<0·001	1·76 (1·00)	1·64 (0·92)	>0·1	<0·001
Hyperlipidaemia	243 (83%)	322 (64%)	<0·001	200 (78%)	335 (65%)	<0·001	>0·1
Smoking‡							
Current	68 (23%)	149 (29%)	<0·001	19 (7%)	41 (8%)	<0·001	<0·001
Ex	178 (61%)	187 (37%)	..	49 (19%)	32 (6%)	..	..
Never	46 (16%)	170 (34%)	..	189 (74%)	444 (86%)	..	..
Nutritional†							
Vitamin B_{12} (pmol/L)	335 (153)	357 (158)	0·06	253 (171)	270 (144)	>0·1	<0·001
Red-cell folate (nmol/L)	446 (196)	385 (166)	<0·001	472 (183)	349 (160)	<0·001	<0·001
Serum folate (nmol/L)	15·8 (8·1)	16·0 (7·6)	>0·1	16·7 (8·3)	15·0 (6·5)	0·002	0·02

Means (SD) for continuous variables and numbers (%) for categorical variables. No variable had more than nine missing values across all groups combined. Hypertension: physician diagnosis of hypertension, or blood pressure >160/90 mm Hg. Diabetes: physician diagnosis of diabetes, or fasting plasma glucose >7·0 mmol/L. Hyperlipidaemia: physician diagnosis of hyperlipidaemia, or serum total cholesterol >5·2 mmol/L.
*Indian Asian versus European controls. †Log transformation used in analysis: geometric mean (approximate SD) reported. ‡Ex denotes at least 6 months cessation. The current and ex categories were combined in derivation of the p presented; p values from univariate analyses: *t* test for continuous variables, χ^2 tests for categorical variables.

Table 1: **Clinical and biochemical characteristics of cases and controls, for Europeans and Indian Asians separately**

of invitation to participate was received by about 1900. There were 1272 replies, of which 1108 attended the hospital for assessment, 83 were excluded based on the prospectively defined criteria, leaving 1025 who completed the study. The response rate was estimated as 54% in Europeans, and 57% in Indian Asians. To ascertain for selection bias in responders compared with non-responders, we reviewed general practice notes for a representative 875 controls. Responders and non-responders, in both ethnic groups, were similar for age, history of hypertension and diabetes, recorded blood pressure and body-mass index, and postal code. Among Europeans, non-responders had a higher prevalence of smoking than responders (40% vs 28%).

Criteria for CHD were: myocardial infarction (chest pain associated with electrocardiographic [ECG] evidence of myocardial infarction or raised cardiac enzymes or both); unstable angina (cardiac pain associated with dynamic ECG abnormalities); angiographically proven coronary artery disease (>50% stenosis in one or more major epicardial vessel in multiple projections). Exclusion criteria for both patients and controls included cardiomyopathy, serious organ disease, systemic illness, chronic alcohol abuse, serious psychiatric illness, anticonvulsant therapy, and—for controls—the presence of pathological Q waves on the ECG. The study was approved by the local ethics committee, and all individuals gave written, informed consent.

Methods

Clinical history, including history of hypertension, diabetes, habitual smoking, alcohol intake, and drug therapy, was recorded in all individuals. Three blood pressure readings were taken by mercury sphygmomanometer, with the individual seated for 10 min, and the mean calculated. Height, weight, and 12-lead ECG were recorded according to standardised protocols.

Samples for plasma homocysteine were taken in the fasting state (overnight), and 6 h after a standard oral methionine load (100 mg/kg), the latter to unmask abnormalities of homocysteine metabolism.[7,8] Samples were placed on ice, centrifuged within 1 h, and the separated plasma stored at −70°C before assays. Additional fasting samples were collected for red-cell folate, serum folate, vitamin B_{12}, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Total plasma homocysteine was measured by high pressure liquid chromatography.[15] Vitamin B_{12} and serum and red-cell folate were measured by radioassay (Simultrac, Becton-Dickenson); lipid profiles were found by an Olympus AU800 multichannel analyser.

Statistical analysis

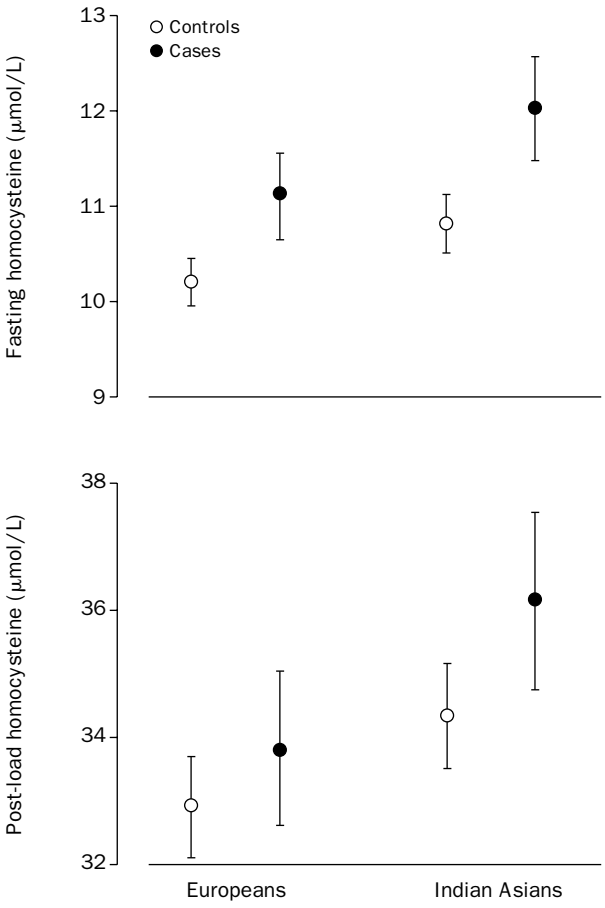
Previous studies in Europeans have reported a 16% (95% CI 12–20) case-control difference in fasting plasma homocysteine, and an SD of fasting (and post-load) homocysteine that was 30–40% of the mean.[8] We initially planned to study 85 cases and 170 controls, in each of the ethnic groups, which offered a 90% power to detect—at a significance level of 5%—a true mean difference of 16% in homocysteine levels between cases and controls.[16] A protocol revision was made in October, 1997, to increase the sample size and power of the study. This change was made after publication of a nested case-control study of homocysteine as a risk factor for CHD in men participating in the Multiple Risk Factor Intervention Trial.[17] In the latter study, the difference in homocysteine concentrations between CHD cases and controls was 1% (−7 to 9), suggesting that the case-control difference in homocysteine was likely to be lower than we had initially anticipated. We therefore increased the sample size of our investigation, and planned instead to recruit 250 cases, and 500 controls in each ethnic group. This offered a 90% power to detect, at a significance level of 5%, a true mean difference of 10% in homocyteine levels between cases and controls, and an 8% difference between European and Indian Asian controls.[16]

We undertook the statistical analysis according to a plan drawn up before the homocysteine results were available. Comparison of cases and controls was done separately for Europeans and Indian Asians. In addition, we compared European controls with Indian Asian controls. A logarithmic transformation of homocysteine concentrations was used to reduce the positive

	Europeans		Indian Asians	
	Cases	Controls	Cases	Controls
Fasting homocysteine	11·1 (3·9)	10·2 (2·9)	12·0 (4·5)	10·8 (3·5)
Post-load homocysteine	33·8 (10·6)	32·9 (8·8)	36·1 (11·1)	34·3 (9·5)
Fasting hyperhomocysteinaemia*	27%	20%	36%	29%
Post-load hyperhomocysteinaemia*	24%	20%	31%	26%

Five values are missing for fasting homocysteine and 34 for post-load homocysteine, across all groups combined.
*Defined according to 80th centile of European control group (fasting >12·4 μmol/L, post load >39·5 μmol/L).

Table 2: **Geometric mean (approximate SD) homocysteine concentrations (μmol/L) in cases and controls, for Europeans and Asians separately**



Geometric mean (95% CI) fasting and post-load homocysteine concentrations in cases and controls, for Europeans and Indian Asians separately

skewness of the distribution, so geometric means are presented; approximate SDs are derived from the variance transformation formula.[18] We used linear regression to estimate the percentage mean case-control difference in homocysteine,[16] with log homocysteine as the dependent variable, and adjusted for other explanatory variables in three stages—first for age; second for blood pressure, glucose, lipids, and smoking (current, ex, or never); third for vitamin B_{12}, red-cell folate and serum folate. In all analyses of post-load homocysteine, adjustment was also made for weight and for fasting homocysteine concentrations. We used logistic regression to estimate the odds ratios of CHD by quintile groups (fifths) of the distribution of homocysteine concentrations in controls, and the odds ratio for a 5 μmol/L increment in homocysteine, adjusted for age alone.

To calculate the magnitude of CHD risk attributable to elevated homocysteine in Indian Asians compared with Europeans, we estimated the CHD population attributable risk within each ethnic group by standard methodology.[12] This was calculated as Pe (RR−1)×100/Pe (RR−1)+1 where RR is the relative risk (derived from previous studies[12]) and P_e the proportion of the population exposed to elevated homocysteine. The number of CHD deaths attributable to elevated homocysteine was then found by multiplying the population attributable risk by age-standardised CHD mortality rate in the two ethnic groups.

Results

Clinical and biochemical characteristics

The characteristics of the study individuals are summarised in table 1. Among controls, Indian Asians had a higher prevalence of hypertension and diabetes, and a lower prevalence of cigarette smoking, than Europeans. In Indian Asian controls, compared with European controls, blood pressure, fasting glucose, and triglycerides were higher, and HDL cholesterol, folate, and vitamin B_{12} lower.

In both ethnic groups, cases were older, had a higher prevalence of hypertension, diabetes, hyperlipidaemia, and current/ex cigarette smoking, compared with controls. Similarly, fasting glucose and red-cell folate were higher, and total and HDL cholesterol lower, in cases compared with controls. European cases had higher body-mass index and triglycerides compared with European controls. Indian Asian cases had higher serum folate, and lower diastolic blood pressure, compared with Asian controls.

Fasting homocysteine concentrations

Fasting homocysteine concentrations were higher in cases compared with respective controls (table 2, figure). After adjustment for age, the case-control difference in fasting homocysteine was 8% (95% CI 3–14), in both Europeans and Indian Asians (p=0·002, table 3). Fasting homocysteine concentrations were positively associated with blood pressure and triglycerides, and negatively associated with vitamin B_{12}, red-cell folate and serum folate (all p<0·001). For both Europeans and Indian Asians, the case-control difference in fasting homocysteine was independent of conventional CHD risk factors, socioeconomic status, and vitamin concentrations (table 3).

The age-adjusted risk of CHD in the top quintile of fasting homocysteine compared with the bottom quintile was 1·5 (95% CI 0·9–2·6) in Europeans, and 1·7 (1·0–2·8) in Indian Asians. The estimated age-adjusted odds ratio of CHD for a 5 μmol/L increase in fasting homocysteine was 1·32 (1·11–1·56) in Europeans and 1·18 (1·03–1·36) in Indian Asians.

Post-load homocysteine concentrations

Post-load homocysteine concentrations were higher in cases compared with respective controls (table 2, figure); the corresponding age-adjusted case-control differences were 4% (95% CI 0–9, p=0·07) in Europeans, and 4% (0–9, p=0·06) in Indian Asians. Post-load homocysteine concentrations were positively associated with fasting homocysteine, blood pressure, and triglycerides, and negatively associated with vitamin B_{12} red-cell folate, and serum folate (all p<0·001). Adjustment of post-load homocysteine for age, weight, and fasting homocysteine concentrations, reduced the case-control difference in homocysteine concentrations to near zero, in both Europeans and Indian Asians (−1% with p>0·05, table 3). This did not change significantly after further adjustment for conventional risk factors and vitamin concentrations (table 3).

Difference in homocysteine concentrations between Europeans and Indian Asians

Fasting homocysteine concentrations were 0·6 μmol/L higher in Indian Asians compared with European controls, corresponding to an age-adjusted difference of 6% (2–10, p=0·002, table 4). After adjusting for folate, the difference was slightly smaller but still significant. The main determinant of the difference in fasting homocysteine between ethnic groups appeared to be vitamin B_{12} concentrations; after adjustment for

Adjustments	Fasting homocysteine		Post-load homocysteine	
	Estimate (95% CI)	p	Estimate (95% CI)	p
Age*				
Europeans	8 (3 to 14)	0·002	−1 (−4 to 3)	0·72
Indian Asians	8 (3 to 14)	0·002	−1 (−4 to 2)	0·67
Conventional risk factors†				
Europeans	9 (2 to 16)	0·01	−2 (−7 to 2)	0·33
Indian Asians	10 (2 to 17)	0·008	2 (−2 to 6)	0·29
Nutritional markers‡				
Europeans	6 (0 to 13)	0·05	−3 (−7 to 2)	0·22
Indian Asians	12 (5 to 19)	0·001	3 (−1 to 7)	0·20

*Post-load homocysteine also adjusted for weight and fasting homocysteine.
†Adjusted for age, blood pressure (systolic pressure, vasodilator therapy), glucose (glucose level, diagnosis of diabetes), lipids (serum total cholesterol, HDL cholesterol, triglycerides, diagnosis of hyperlipidaemia), and smoking (current, ex, or never):
‡Adjusted in addition to conventional risk factors, for vitamin B_{12}, red-cell folate, and serum folate.

Table 3: **Percentage difference in homocysteine concentrations between cases and controls, for Europeans and Indian Asians separately**

vitamin B_{12} the difference was much closer to zero. Adjustment for other factors had little additional effect (table 4).

The average difference in post-load homocysteine concentrations between the European and Indian Asian controls was 1·4 μmol/L. After adjustment for age, weight, and fasting homocysteine concentrations, the difference in post-load homocysteine concentrations was reduced to 1% (−1 to 4, p=0·35, table 4), and further adjustment for folate and vitamin B_{12} had little effect (table 4).

CHD risk attributable to homocysteine in Indian Asians

We calculated both the absolute risk of CHD in the two ethnic groups, and the proportion of the excess CHD risk in Indian Asians, that was attributable to elevated homocysteine.

The CHD population attributable risk, and the number of CHD deaths attributable to homocysteine in Europeans and Indian Asians are summarised in table 5. The CHD population attributable risk from homocysteine was 7·2% in Europeans, and 10·4% in Indian Asians. On the basis of the age-standardised annual CHD mortality rates for European and Indian Asian men (199 and 274 per 100 000 population respectively),[1] these correspond to 140 and 280 CHD deaths per year in European and Indian Asian men respectively, per million population. Elevated homocysteine may therefore contribute to about twice as many deaths in Indian Asian, compared with European men.

Age-adjusted fasting homocysteine concentrations were 6% (2–10) higher in Indian Asians, compared with Europeans. On the basis of a previous meta-analysis, we would expect this to lead to a 6% increase in CHD risk.[12]

	Fasting homocysteine		Post-load homocysteine	
	Estimate (95% CI)	p	Estimate (95% CI)	p
Adjustments				
Age	6 (2 to 10)	0·002	1 (−1 to 4)	0·35
Age, red cell folate, serum folate	4 (1 to 8)	0·02	1 (−1 to 4)	0·35
Age, vitamin B_{12}	2 (−1 to 6)	0·22	1 (−1 to 4)	0·43
Age, vitamin B_{12}, red-cell folate, serum folate, glucose, triglycerides	1 (−2 to 5)	0·48	1 (−1 to 4)	0·40

Post-load homocysteine concentrations also adjusted for weight, and fasting homocysteine concentrations.

Table 4: **Percentage difference in homocysteine concentrations, for controls alone, in Indian Asians compared with Europeans**

	Proportion of population exposed		Odds ratio for CHD*	Population attributable risk	
	Europeans	Indian Asians		Europeans	Indian Asians
Homocysteine concentration (μmol/L)					
<11	66·1%	55·6%	..	..	..
11–12	10·3%	11·4%	1·07	0·71%	0·79%
12–13	8·5%	8·5%	1·15	1·26%	1·26%
13–14	5·3%	7·3%	1·24	1·26%	1·73%
14–15	2·0%	4·8%	1·33	0·65%	1·57%
⩾15	7·9%	12·4%	1·43	3·28%	5·04%
Total population attributable risk	..	..	..	7·2%	10·4%
Age-standardised annual CHD mortality rate[1]	..	..	..	199	274
CHD deaths annually/100 000 men	..	..	..	14	28

*The odds ratio of CHD for each homocysteine interval.[12]

Table 5: **Deaths from CHD attributable to elevated homocysteine concentrations in Europeans and Indian Asians**

Hence, of the reported 40% excess risk of CHD in Indian Asians compared with Europeans in the age group entered into this study,[1] about 17% (log 1·06/log 1·40) may be attributable to homocysteine. Taking into account regression dilution bias,[19,20] the proportion of the excess CHD risk in Indian Asians compared with Europeans that may be attributable to elevated homocysteine concentrations is estimated as 20%. However, this figure cannot be considered exact, since there is imprecision in each of the components of the calculation.

Discussion

We have shown that plasma homocysteine is a novel and independent risk factor for CHD in UK Indian Asians. In our study, plasma homocysteine concentrations were higher in Indian Asians, compared with Europeans, and the differences were explained by reduced concentrations of vitamin B_{12} and folate in Asians. We propose that plasma homocysteine contributes to the increased CHD risk in Indian Asians, which may be lowered by dietary vitamins.

Plasma homocysteine is increasingly recognised as a risk factor for CHD in North American and European populations.[7–10] In this study, fasting homocysteine concentrations were higher in Indian Asian cases compared with controls. The association between fasting plasma homocysteine and CHD was independent of conventional risk factors including diabetes and metabolic disturbances associated with insulin resistance. The odds ratio of CHD for a 5 μmol/L increment in fasting plasma homocysteine was 1·2 (95% CI 1·0–1·4) in Indian Asians, and 1·3 (1·1–1·6) in Europeans. Our results are consistent with the odds ratio of 1·6 (1·4–1·7) for CHD reported in previous studies in Europeans.[12,21] Post-load homocysteine was not significantly associated with CHD, after adjustment for fasting homocysteine concentrations. This observation suggests to us that post-load homocysteine provides little more information than measurement of fasting homocysteine concentrations alone.

The precise mechanisms that underlie the increased CHD mortality rates in Indian Asians are not clear. In our study, the prevalence of conventional coronary risk factors, diabetes, and metabolic disturbances associated with insulin resistance among Indian Asians, was similar to that reported elsewhere.[2,3] Previous studies suggest that these risk factors do not completely explain the higher CHD risk in Indian Asians, compared with Europeans.[2,3,5] In our study, mean fasting plasma homocysteine

concentrations were 6%, (2–10) higher in Indian Asians than in Europeans. We have assessed the burden of CHD mortality attributable to homocysteine in Indian Asians and Europeans, and we calculate that raised homocysteine concentration may account for twice as many CHD deaths in Indian Asians compared with Europeans. These findings suggest an important contribution of homocysteine to the increased CHD risk of Indian Asians.

Homocysteine concentrations are determined by genetic and nutritional factors,[6] including deficiencies of folate, and vitamins B_{12} and B_6. Reduced intake of vitamin B_{12} has been reported in Indian Asians, and prolonged cooking of vegetables, which is common practice in many Indian Asian households, may destroy up to 90% of folate content.[13,14,22] In our study, differences in homocysteine concentration between the two ethnic groups were explained by lower serum B_{12} and folate concentrations in Indian Asians, suggesting that nutritional factors may underlie elevated plasma homocysteine concentrations in this group. Although intervention studies need to be undertaken, dietary supplementation with vitamins to lower homocysteine concentrations[23] may provide a simple, effective, and inexpensive means of reducing CHD risk in Indian Asians.

In conclusion, our results provide evidence that elevated plasma homocysteine concentrations are a novel independent risk factor in Indian Asians that may account for an important proportion of their increased CHD risk. Our results provide the basis of future intervention studies aimed at reducing homocysteine concentrations with dietary vitamins in this high risk ethnic group.

Contributors

John C Chambers and Jaspal S Kooner were responsible for the study design, recruitment and characterisation of patients, data entry, and statistical analyses. Omar A Obeid, Helga Refsum, Per Ueland, and James Hooper undertook the laboratory assays. David Hackett was responsible for study design, and pilot data. Rebecca M Turner did statistical analyses. Simon G Thompson was responsible for study design, planning, and conduct of the statistical analyses. All investigators contributed to the preparation of the paper.

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