Plasma Homocysteine as a Risk Factor for Vascular Disease

The European Concerted Action Project

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Context.—Elevated plasma homocysteine is a known risk factor for atherosclerotic vascular disease, but the strength of the relationship and the interaction of plasma homocysteine with other risk factors are unclear.

Objective.—To establish the magnitude of the vascular disease risk associated with an increased plasma homocysteine level and to examine interaction effects between elevated plasma homocysteine level and conventional risk factors.

Design.—Case-control study.

Setting.—Nineteen centers in 9 European countries.

Patients.—A total of 750 cases of atherosclerotic vascular disease (cardiac, cerebral, and peripheral) and 800 controls of both sexes younger than 60 years.

Measurements.—Plasma total homocysteine was measured while subjects were fasting and after a standardized methionine-loading test, which involves the administration of 100 mg of methionine per kilogram and stresses the metabolic pathway responsible for the irreversible degradation of homocysteine. Plasma cobalamin, pyridoxal 5'-phosphate, red blood cell folate, serum cholesterol, smoking, and blood pressure were also measured.

Results.—The relative risk for vascular disease in the top fifth compared with the bottom four fifths of the control fasting total homocysteine distribution was 2.2 (95% confidence interval, 1.6-2.9). Methionine loading identified an additional 27% of at-risk cases. A dose-response effect was noted between total homocysteine level and risk. The risk was similar to and independent of that of other risk factors, but interaction effects were noted between homocysteine and these risk factors; for both sexes combined, an increased fasting homocysteine level showed a more than multiplicative effect on risk in smokers and in hypertensive subjects. Red blood cell folate, cobalamin, and pyridoxal phosphate, all of which modulate homocysteine metabolism, were inversely related to total homocysteine levels. Compared with nonusers of vitamin supplements, the small number of subjects taking such vitamins appeared to have a substantially lower risk of vascular disease, a proportion of which was attributable to lower plasma homocysteine levels.

Conclusions.—An increased plasma total homocysteine level confers an independent risk of vascular disease similar to that of smoking or hyperlipidemia. It powerfully increases the risk associated with smoking and hypertension. It is time to undertake randomized controlled trials of the effect of vitamins that reduce plasma homocysteine levels on vascular disease risk.

JAMA. 1997;277:1775-1781

CASE-CONTROL and prospective studies indicate that an elevated plasma homocysteine level is a powerful risk factor for atherosclerotic vascular disease. The relationship has been reported for elevated plasma homocysteine levels measured with the subject fasting and after a methionine-loading test. Despite the consistency of these reports, methodological problems make it difficult to be certain of the strength of the relationship and in particular of the independence of or interactions between elevated plasma homocysteine level and conventional risk factors. Elucidation of these issues is an essential part of testing the hypothesis that the relationship between elevated plasma homocysteine level and vascular disease is causal. The presence of interaction effects might modify current approaches to vascular disease prevention. In addition, few studies to date have examined the risk associated with elevated plasma homocysteine levels in women. Plasma homocysteine levels are determined by both genetic and nutritional factors. Of the latter, supplementation with folic acid in particular can reduce homocysteine levels. This observation may have substantial public health implications.

With the use of the European Community Concerted Action structure, a case-control study was designed with the following objectives: (1) to examine the relationships between plasma total homocysteine (tHcy) and vascular disease risk in male and female European subjects; (2) to study possible interaction effects between plasma tHcy level and conventional risk factors; and (3) to establish whether the blood levels of the nutrients that modulate plasma tHcy levels (red blood cell folate, cobalamin, and pyridoxal phosphate) relate to vascular disease. In this report, we concentrate on the first 2 objectives.
Plasma tHcy level was analyzed as a categorical variable. An elevated tHcy level was defined as one in the top fifth of the control distribution (fasting, >12 μmol/L; postload, >38 μmol/L). The increase in tHcy level (postload minus the fasting value) also had a cutoff point defined by the top fifth of the control distribution (≥27 μmol/L). Relative risks were estimated with those in the bottom four fifths of the distribution used as reference.

Because of the large number of treated hypertensive patients and the effect of treatment on actual levels, we also analyzed blood pressure as a categorical variable. Hypertension was deemed to be present if the mean of the 4 readings was 160 mm Hg or more (systolic) or 95 mm Hg or more (diastolic), or the subject was taking antihypertensive medication.

Smoking was analyzed as a continuous variable in terms of the current total amount of tobacco smoked expressed in equivalent number of cigarettes per day. Relative risks for smoking were based on a comparison between the risk for a smoker of exactly 20 cigarettes per day compared with a nonsmoker.

Serum cholesterol level was also analyzed as a continuous variable, and relative risks were based on a comparison of risks in subjects at the top and bottom quintiles of the cholesterol distribution in controls, 7.1 mmol/L (275 mg/dL) and 4.9 mmol/L (189 mg/dL), respectively.

Each case was categorized as one of coronary heart, cerebrovascular, or peripheral vascular disease. If a case had several diagnoses, the major manifestation was used.

**Statistical Methods**

Logarithmic transformations and geometric means were used for variables showing a marked positive skew. Analysis of variance, least-squares regression, and t test and χ² test were used for initial data analysis with 2-tailed levels of significance.

Risk analyses were performed by means of conditional logistic regression for all vascular disease and for the 3 subcategories separately. All of the controls were used for each category, and all risk analyses were stratified by age group (<40, 40-49, and ≥50 years), sex where relevant, and the 19 centers. Odds ratios, which are described in this article as relative risks, together with their 95% confidence intervals (CIs), were derived from the regression coefficient with dummy variables used for the dose-response analysis.

Two risk factors interact with each other if the joint effect of the 2 factors in combination is different from that expected on the basis of their separate effects. Two models may be used to determine interaction—the additive and the multiplicative—but without a great deal of knowledge about the processes that lead to disease, it is impossible to know which model is more appropriate. The multiplicative model tends to be the more commonly used because it is the model underlying logistic regression, but there have been recent suggestions that cardiovascular risk factors may not act multiplicatively.

For the calculation of the observed relative risk in those exposed to 2 factors, a multiplicative interaction term was used.
was included in the logistic model even if it was statistically nonsignificant, thus avoiding the otherwise automatic acceptance of a multiplicative model. The expected joint relative risks, under both the multiplicative and additive models, were based on the individual relative risks for each factor on its own. Significance of departure from a particular model was based on hypothesis tests on the regression coefficients. The interaction analysis employed a model that included either fasting or postload tHcy level, the 3 conventional risk factors (cholesterol, smoking, and blood pressure), and the 3 interactions between the risk factors and the particular tHcy level, with age, sex, and center as stratification variables.

The need to examine interaction effects between tHcy and the 3 conventional risk factors necessitated a larger sample than that of earlier studies. The chosen sample size was based on a particular quantification of an interaction between 2 continuous variables and is described elsewhere.16

RESULTS

Case-Control Differences

Cases were slightly older, had higher plasma tHcy, cholesterol, triglyceride, and systolic blood pressure levels, and used more tobacco than controls (P<.001). Serum cobalamin levels were similar in cases and controls. Red blood cell folate levels were nonsignificantly lower and serum pyridoxal phosphate levels significantly lower in cases than in controls (Table 2). Cases were significantly less likely than controls to be taking supplements containing these vitamins. Users of supplements containing folic acid, cobalamin, or pyridoxine had a relative risk of 0.88 (95% CI, 0.82-0.96) compared with nonusers (adjusted for conventional risk factors).

Geometric mean fasting tHcy levels were 16% (95% CI, 12%-20%) and postload tHcy levels 17% (95% CI, 13%-21%) higher in cases than in controls. Similar differences were noted in each vascular disease category. Arithmetic mean tHcy levels were 20% higher in cases for both fasting and postload values. The case-control differences were explained by a shift in the case distribution to the right with a more pronounced positive skew in cases (Figure 1). In controls, fasting tHcy levels were significantly and independently higher in older subjects and in men. Postload levels showed a similar association with age but not sex. Despite variation in tHcy levels between centers, no clear geographical trend was apparent, perhaps because of small numbers in some southern European centers. The increase in plasma tHcy level (postload minus fasting) was also significantly higher in cases than in controls (Table 2).

Relationships between fasting and postload total tHcy level were also examined. Although the measures were highly correlated (Figure 2), different persons with elevated tHcy levels were identified by each measure. A total of 30.1% (241/800) of controls had elevated tHcy levels, either fasting, postload, or both, compared with 50% (375/750) of cases; 13.5% (101/750) of all cases (26.9% [101/375] of cases with elevated tHcy levels) would not have been so classified if the postload estimation had not been available (Figure 3). An elevated increase in plasma tHcy level was defined as an increase (postload minus fasting) above the top quintile in controls. A further 18 controls (2.3%) and 18 cases (1.7%) with elevated increases who did not have elevated fasting or postload levels were identified by this additional measure.

Vascular Disease Risk and Elevated Plasma tHcy Levels

In the univariate analysis using present definitions, both elevated fasting and postload tHcy levels were strongly related to vascular disease and
but this was not statistically significant.

The simultaneous effect on vascular disease of an elevated fasting, an elevated postload, and an elevated increase in tHcy levels was examined in a single logistic regression model (adjusting for the 3 classic risk factors). The results are presented in Figure 3. Although an elevated increase in tHcy level identified an extra 18 controls and 13 cases who did not have elevated fasting or postload levels, there was no independent effect on risk associated with an elevated increase in tHcy. On the other hand, an elevated postload level contributed independently to risk. For risk analyses, therefore, the effects of an elevated increase in tHcy level are not considered further. Fasting and postload elevations in tHcy had independent effects on risk and had a multiplicative effect when present together. The relative risk for a subject with an elevated fasting tHcy level only was 1.6 (95% CI, 1.0-2.2) and that for an elevated postload level only was 1.5 (95% CI, 1.0-2.2), while for a subject with both elevated fasting and postload levels, the relative risk was 2.5 (95% CI, 1.7-3.5).

Figure 4 demonstrates the possibility of a dose-response effect between tHcy level and vascular disease risk. Risk begins to rise from the middle of the distribution, with the increase most apparent beyond the eighth decile (top quintile). For this reason, the top fifth of the control distribution was compared with the bottom four fifths in deriving relative risks. Comparing the top 10th with the bottom 10th, subjects had a relative risk of 3.1 (95% CI, 1.9-5.2) for fasting tHcy and 3.7 (95% CI, 2.2-6.1) for postload tHcy. When tHcy was considered as a continuous variable, the relative risk per 5-μmol/L increment in fasting tHcy level was 1.35 (95% CI, 1.1-1.6) for men and 1.42 (95% CI, 0.99-2.06) for women.

Interactions Between Plasma tHcy Level and Other Risk Factors

Figure 5 and Table 4 show the relative risks in various combinations of tHcy levels and the conventional risk factors. In men, elevated fasting tHcy level showed an additive effect with cholesterol level and greater than multiplicative interaction with smoking and blood pressure. Elevated postload tHcy level produced interaction effects that were greater than multiplicative with cholesterol level and greater than additive with smoking and blood pressure. Similar interaction effects were apparent in wom-

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**Figure 1.**—Distribution of fasting and postload plasma total homocysteine levels in vascular disease cases (n=750) and controls (n=800).

**Figure 2.**—The association between fasting and postload plasma total homocysteine levels in all vascular disease cases and controls. The reference lines are at the control quintile cutoff points used to define elevated values. The percentages in circles are the proportion of controls (or cases) with combinations of elevated fasting and postload tHcy levels. The percentages in diamonds are the proportion of controls (or cases) with combinations of an elevated fasting and postload tHcy level.

**Figure 3.**—Numbers of subjects (controls/cases) with elevated fasting, elevated postload, and elevated increase of plasma total homocysteine level with associated relative risks (shown in large bold type) of vascular disease (adjusted for age, sex, center, and other risk factors). Relative risks for all subjects with elevated fasting, elevated postload, or elevated increase in total homocysteine level are shown separately (last line of type).

**Figure 4.**—The association between fasting and postload plasma total homocysteine levels in all vascular disease cases and controls. The reference lines are at the control quintile cutoff points used to define elevated values. The percentages in circles are the proportion of controls (or cases) with combinations of elevated fasting and postload tHcy levels. The percentages in diamonds are the proportion of controls (or cases) with combinations of an elevated fasting and postload tHcy level.
en and in many of the individual vascular disease categories.

COMMENT

This study addresses the strength and possible independence of the relationship between plasma tHcy level and all categories of atherosclerotic vascular disease. The design and power of the study were sufficient to allow examination of interaction effects between tHcy level and conventional risk factors. These relationships may have considerable implications for public health.

In the present study, subjects with plasma tHcy levels in the top fifth of the control distribution, either fasting or after methionine loading, had a 2-fold increase in vascular disease risk compared with the remaining four fifths. This level of risk was equivalent to that of hypercholesterolemia or smoking and applied to all categories of vascular disease. It is lower than earlier estimates of risk because of the lower cutoff point chosen to define elevated tHcy level and for this reason applies to a much larger proportion of the population. The risk estimate was also independent of the effect of other risk factors.

Fasting and postload tHcy levels were highly interrelated. The former may reflect cobalamin- and folate-dependent remethylation, and the latter, pyridoxal 5′-phosphate-dependent transsulfuration. Reliance on fasting tHcy level alone will result in classifying 27% fewer patients as hyperhomocysteinemic. Both and colleagues reported that use of fasting tHcy level alone would have led to classification of more than 40% fewer subjects as being hyperhomocysteinemic.

The risk associated with elevated post-load tHcy was higher in women, particularly those with coronary heart disease. While not statistically significant, this finding may be clinically relevant if confirmed in larger numbers of subjects. It is possible that the age cutoff point of 60 years selected particularly high-risk women. A similar effect might partially reflect cobalamin- and folate-dependent remethylation, and the latter, pyridoxal 5′-phosphate-dependent transsulfuration. Reliance on fasting tHcy level alone will result in classifying 27% fewer patients as hyperhomocysteinemic. Both and colleagues reported that use of fasting tHcy level alone would have led to classification of more than 40% fewer subjects as being hyperhomocysteinemic.

The strong, independent association between elevated tHcy level and vascular disease risk is consistent with other

Figure 4.—Relative risks of vascular disease in groups defined by deciles (in controls) of fasting and postload plasma total homocysteine level with 95% confidence intervals (adjusted for age, sex, center, cholesterol, smoking, and blood pressure).
tween elevated plasma homocysteine levels and vascular disease, randomized controlled trial evidence of benefit arising from homocysteine reduction is lacking.

Plasma homocysteine concentration relates inversely to blood levels of folate, cobalamin, and pyridoxine and to intakes of these vitamins. The present study confirms this observation (data not shown). In this study, users of vitamin preparations containing these vitamins appear to experience substantial protection from vascular disease, with a relative risk of 0.38 (95% CI, 0.2-0.72) compared with nonusers of vitamins. However, this observation applies to a small number of subjects who may have been more health conscious in other ways and cannot be taken as proof of benefit of the known homocysteine-lowering effect of these nutrients.

Interactions between plasma tHcy level and conventional risk factors may have implications for risk management and for our understanding of the causes of vascular disease. The joint effect of smoking and elevated plasma tHcy level was compatible with a multiplicative interaction effect in both sexes combined. An even stronger interaction between elevated fasting tHcy level and hypertension was noted, especially in women. It is conceivable that tHcy may augment smoking-related platelet and clotting effects or exert a toxic effect on the endothelium, and these might be more relevant to the genesis of vascular disease than reported effects of homocysteine and lipoprotein oxidation. Control of smoking and hypertension may be particularly important in subjects with elevated tHcy levels, and estimation of plasma tHcy should now be considered as part of total vascular disease risk assessment.

We conclude that an elevated plasma tHcy level is now established as a strong and independent factor associated with all categories of atherosclerotic disease in both men and women. An elevated plasma tHcy level interacts strongly with smoking and hypertension. It is known that folate supplementation reduces homocysteine levels both in the fasting state and after methionine loading, and that pyridoxal 5'-phosphate can lower postmethionine homocysteine levels. Users of these vitamins have lower homocysteine levels than nonusers do, and there is a suggestion of reduced risk in vitamin users in the present study. We believe it is time to consider whether existing recommended daily allowances of the vitamins that modulate homocysteine metabolism are adequate, and to undertake randomized controlled trials of the effects of folic acid and perhaps pyridoxine in the secondary prevention of cardiovascular disease.
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This study was supported by grants MR4/0829/IHL and MR4/0828/IHL from the European Community and by grants from the following bodies: the Irish Heart Foundation; the Irish Health Research Board; the Norwegian Council on Cardiovascular Diseases; the Norwegian Research Council; the Norwegian Society for Science and the Humanities; the Netherlands Organisation for Scientific Research; the Foundation for Metabolic Research of Utrecht; the Northern Ireland Chest; Heart and Stroke Association of Ontario; Instituto Nazionale di Prevenzione e Diagnostico Sanitarie della Seguridad Social (FIS), Madrid, Spain; Progetto strategico "Farmaci per malattie cardiovascolari" nell'ambito del sottoprogetto Omocistinuria, Rome, Italy; Consiglio Nazionale delle Ricerche, Rome; S.A. Roche, Brussels; the Swedish Medical Research Council; and The Swedish Medical Research Council.

We wish to acknowledge the intellectual and professional expertise of the following individuals in the planning and execution of this concerted action project: Paul Boissieras, PhD; David Bouchier-Hayes, FRCS; Geoffrey Bourke, MD, FRCP; Seamus Cahalane, MD, FRCP; Francois Cambien; Robert Clarke, MD; Fulvio Facchini, MD; Martin Feely, FRCSI, Brian Fowler, PhD; Rafael faella de Franchis, MD; Michael Gibney, PhD; Anders Green, MD; Arne Hamfelt, PhD; Noel Hickey, MD, FRCP (deceased); Frans Kok, PhD; Marcel Kornitzer, PhD; Jan Kraus, PhD; Vincent Maes, PhD; Michael McPartlin, PhD; Joseph di Minno, PhD; Luis Cayollia de Motta, MD (deceased); Eileen Naughten, FRCP; Luke O'Donnell, MD, FRCP; Jesus de Pedro Cuesta, MD, PhD; Luca Raineri, MD; Susanna Sama, MD; Emer Shelley, MD, Cairns Smith, MD; Sander Stokroo; Pietro Straciuglio, MD; Leslie Taylor; Michael Walsh, MD, FRCP; Noeljine Walsh; Ursula White; Steven Whitehead, DPhil; John Yarnell, MD. Thanks are also due to P. H. Rolland, F. Ambrosi, Bariatier, P. Beloucha, M. Chatei, and R. Lucion. Special thanks are due to Betty Turner for her organizational skills, to Linda O’Connell for her patience in typing the manuscript, and to Arthur Ogilvie for photographic work.

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