See corresponding Perspective on page 333.

The controversy over homocysteine and cardiovascular risk[1,2]

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ABSTRACT Elevated plasma total homocysteine (tHcy) is a risk factor for occlusive cardiovascular disease (CVD). This concept is based on the observations of premature vascular disease in patients with homocystinuria, the relation between tHcy and both clinical CVD as well as preclinical atherosclerotic disease, the relation between tHcy in children and CVD in their parents or relatives, and reduction in CVD or surrogate endpoints after tHcy-lowering intervention with B vitamins. Plausible mechanisms include the in vivo interference with nitric oxide–dependent reactive vasodilatation. Some observations have raised questions about tHcy as a risk factor. *1*) Some prospective studies showed a weak relation or no relation between tHcy and CVD. *2*) Several traditional risk factors are associated with tHcy and may confound the relation between tHcy and CVD. *3*) tHcy is related to renal function, and hyperhomocysteinemia may reflect early nephrosclerosis. *4*) The C677T transition of the methylenetetrahydrofolate reductase gene causes a moderate increase in tHcy but no or only minor increased CVD risk. However, the strength of some of these arguments can be questioned because there is increasing evidence that tHcy is a proximate risk factor provoking the acute event, it strongly interacts with traditional risk factors, and it may predict CVD or death in patients with chronic renal failure. Furthermore, the studies of the C677T polymorphism lack statistical power, and the *TT* genotype may even modulate CVD risk independently of homocysteine. Thus, only placebo-controlled intervention studies with tHcy-lowering B vitamins and clinical endpoints can provide additional valid arguments for the debate over whether tHcy is a causal CVD risk factor. *Am J Clin Nutr* 2000;72:324–32.

KEY WORDS Homocysteine, cardiovascular disease, methylenetetrahydrofolate reductase polymorphism, renal, nephrosclerosis

INTRODUCTION

Patients with the inborn metabolic error homocystinuria have markedly elevated homocysteine concentrations in plasma and urine and occlusive vascular disease in early adulthood or even in childhood (1). On the basis of these observations, McCully (2) formulated the homocysteine theory of atherosclerosis in 1969 (2). In 1976, Wilcken and Wilcken (3) published their pioneering work on abnormal homocysteine metabolism in patients with coronary artery disease. Since then, convincing evidence has been gathered on the relation between moderate elevation of plasma total homocysteine (tHcy) and the risk of occlusive vascular disease in the coronary, cerebral (4), and peripheral arteries and, more recently, of venous thrombosis (5–7). The literature on this subject now includes >120 articles reporting on >12 000 patient–control subject sets. Almost all of the retrospective case-control studies and most of the prospective studies support the concept of hyperhomocysteinemia as a risk factor for cardiovascular disease (CVD; 6, 8), and several meta-analyses showed similar, consistent results, as summarized in **Figure 1**.

Some observations may suggest that elevated tHcy is an epiphenomenon secondary to the vascular disease itself (13, 14). In this article, we briefly discuss these arguments but focus on the evidence that hyperhomocysteinemia is an antecedent phenomenon that may provoke the vascular lesion.

PROSPECTIVE STUDIES

To date, >20 prospective studies of the topic have been published (6, 8, 15). Among these, the population-based, nested, case-control studies showed that a 5-μmol/L increment in tHcy results in a 20–30% increase in cardiovascular risk, which is substantially lower than the 60–90% risk enhancement shown in the retrospective case-control studies (Figure 1) (6, 8, 15). The prospective studies also suggested that the risk is highest during short-term follow-up and is attenuated after 3–4 y (16, 17). Notably, tHcy is a particular strong predictor of cardiovascular events or death in subjects with preexisting illness, such as renal failure (18), coronary heart disease (19), peripheral artery disease (20), diabetes (21), systemic lupus erythematosus (22), and venous thromboembolism (23). In line with this, one study showed that high tHcy is more strongly associated with recurrence of an event than with first-ever stroke or myocardial infarction (24). From these observations, one may infer that hyperhomocysteinemia is particularly deleterious in subjects with an underlying disease and that it affects the short-term outcome in these patients.

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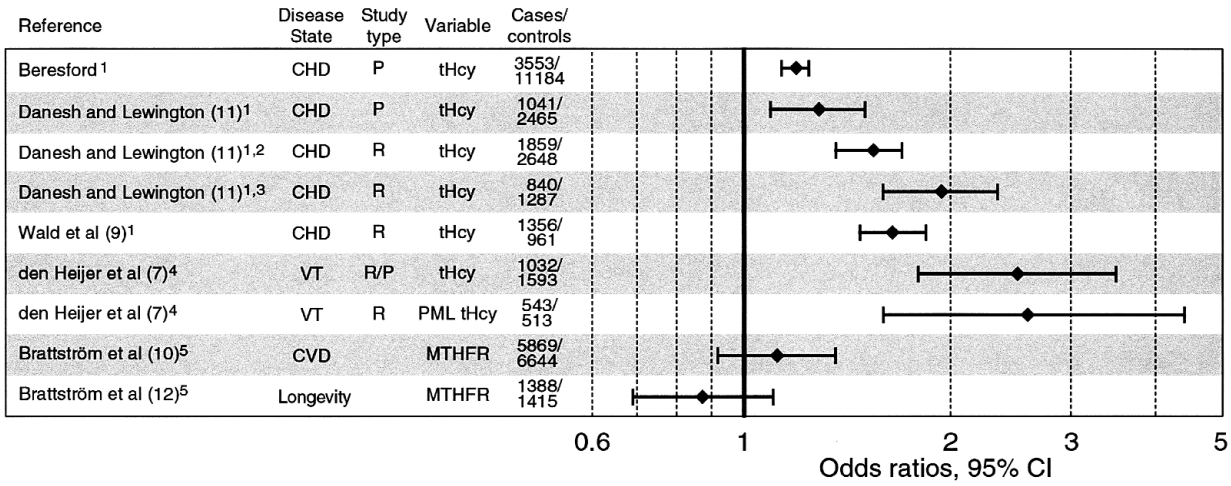

FIGURE 1. Odds ratios (OR) with 95% CIs for the prediction of cardiovascular disease (CVD) and death by homocysteine and the methylenetetrahydrofolate reductase (MTHFR) genotype obtained in 9 meta-analyses. CHD, coronary hear disease; VT, venous thrombosis; P, prospective; R, retrospective; tHcy, total homocysteine; PML, post–methionine load. Our meta-analysis is presented in Table 1. Beresford is a member of Ueland et al's group (this article).
[1]OR for a 5-μmol increase in tHcy.
[2]Population-based controls.
[3]Other controls.
[4]OR for elevated tHcy [above the 95th percentile of controls or the mean plus 2 (or 2.7) SD].
[5]OR for *TT* compared with *CC* genotype.

UPDATED META-ANALYSIS OF PROSPECTIVE STUDIES ON HEART DISEASE

We updated our meta-analysis (25, 26) by including articles on MEDLINE through October 1999. Most studies evaluated the association between homocysteine concentrations and risk of coronary heart disease while adjusting for age, smoking status, blood pressure, and serum cholesterol. Several studies adjusted for additional factors such as body mass index, diabetes, and physical activity. For all studies, we calculated or estimated the risk per 5-μmol/L change in homocysteine concentration. In some instances, we calculated the regression coefficient from the mean by using the linear discriminant function method (27). In other instances, we estimated the regression coefficient for an extreme quantile contrast and then applied it to a distance of 5 μmol/L by using linear interpolation. To calculate the pooled odds ratio (OR), we used the general variance-based method (28). We identified 14 relatively recent prospective studies. Of these, 9 provided information specific to men and 6 provided information specific to women. The resulting pooled OR per 5-μmol/L change in tHcy was 1.13 (95% confidence limits: 1.07, 1.19) for men and 1.61 (1.34, 1.92) for women. Within the group of 4 studies that reported results for each sex separately, the pooled OR for men was not significantly different from that for women. We therefore combined the results for men and women from these studies. Each of the 14 prospective studies contributed one OR, as shown in **Table 1**. Pooling these, the estimated OR for coronary heart disease for a 5-μmol/L increase in homocysteine was 1.20 (1.14, 1.25).

The results of prospective studies confirm the findings of previous meta-analyses, dominated by retrospective case-control studies, that the tHcy concentration is significantly associated with the risk of coronary heart disease (Figure 1). The pooled estimates for these prospective studies were somewhat smaller than those from population-based case-control studies, which for men estimated an increased risk on the order of 1.5 or 1.6 (Figure. 1; 11). Several of the prospective studies had long periods of follow-up (>10 y). If elevated homocysteine is a proximate risk factor provoking the acute event (16, 34), this risk might be attenuated over a longer follow-up. For these reasons, the pooled estimates from prospective studies are not inconsistent with the pooled estimates from case-control studies. Note that our most conservative approach to estimating the ORs (assuming a linear relation and using ORs after adjusting for multiple variables without correcting for the regression-dilution bias) still results in an elevated OR for coronary heart disease.

STUDIES IN CHILDREN

There are consistent reports that high plasma tHcy in children is related to CVD or death in their parents or close relatives (40–42). This was shown in white and black children and in white children with hypercholesterolemia. In the latter study group, the methylenetetrahydrofolate reductase (MTHFR) *TT* genotype tended to be most frequent in children with a parental history of CVD (43). Because genetic and environmental factors determining tHcy may be shared within a family, elevated tHcy may partly explain the increased risk related to a family history of CVD. These facts certainly weaken the possibility that the association between tHcy and CVD is secondary to the acute event or reflects preclinical vascular pathology.

INTERACTIONS WITH CONVENTIONAL RISK FACTORS

The idea that elevated tHcy has a negative effect on the short-term outcome of patients with preexisting disease agrees with the observation that hyperhomocysteinemia interacts with other cardiovascular risk factors. This hypothesis was addressed in the

TABLE 1
Meta-analysis of prospective studies of total homocysteine (tHcy) and coronary heart disease[1]

Reference	Study	Sex	Age range	Cases	Controls	OR (95% CI) per 5-μmol/L tHcy increment
			y			
Alfthan et al (30)[2]	Finland	M, F	40-64	191	269	1.03 (0.66, 1.53)
Arnesen et al (31)	Tromsø	M, F	34–61	122	478	1.41 (1.06, 1.88)
A'Brook et al (29)[2,3]	Scotland	M, F	35-64	335	335	1.50 (1.28, 1.78)
Bostom et al (32)[4]	Framingham	M, F	59–91	244	1933[9]	1.42 (1.13, 1.77)
Bots et al (33)[5]	Rotterdam	M, F	≥55	104	533	1.28 (1.05, 1.76)
Evans et al (34)[6]	MRFIT	M	35–57	227	414	0.98 (0.83, 1.15)
Folsom et al (35)[5]	ARIC	M, F	45–64	232	537	1.15 (0.68, 1.92)
Kark et al (17)[7]	Jerusalem	M, F	≥50	135	1788[9]	1.34 (1.05, 1.62)
Ridker et al (36)[5]	Women's Health Study	F	Postmenopausal	85[8]	170	1.74 (1.13, 2.64)
Stampfer et al (37)	Physician's Health Study	M	40–84	271	271	1.29 (1.01, 1.64)
Stehouwer et al (24)	Zutphen	M	64-84	98	780	1.05 (0.97, 1.15)
Ubbink et al (38)[5]	Caerphilly	M	50–64	154	2136	1.22 (0.88, 1.64)
Wald et al (9)	BUPA	M	35–64	229	1126	1.41 (1.20, 1.65)
Whincup et al (39)[7]	British Regional Heart Study	M	40–59	359[8]	414[8]	1.13 (0.99, 1.29)
Pooled OR						1.20 (1.14, 1.25)

[1] ORs calculated from the data provided in the articles. MRFIT, Multiple Risk Factor Intervention Trial; ARIC, Atherosclerosis Risk in Communities; BUPA, British United Provident Association.
[2] OR calculated by using the linear discriminant function method.
[3] Abstract only.
[4] OR calculated from upper quartile contrast.
[5] OR calculated from extreme quintile contrast.
[6] OR calculated from extreme quartile contrast.
[7] OR calculated from 1 SD of ln scale.
[8] Preexisting coronary heart disease not excluded.
[9] *n* in the total cohort.

European COMAC project on homocysteine and vascular disease (44). This case-control study of 750 CVD patients and 800 control subjects showed that hyperhomocysteinemia had a more than multiplicative effect on risk in smokers and hypertensive subjects and also enhanced the risk conferred by elevated cholesterol. A strong effect modification of the tHcy-CVD association by conventional risk factors may also explain the recent observation that plasma tHcy is not related to coronary heart disease in patients without conventional risk factors such as hypertension, diabetes, and hyperlipidemia (45).

Hyperhomocysteinemia may also interact with the genetic predisposition to thrombosis, as was recently shown for the factor V Leiden mutation. The combined presence of these 2 risk factors conferred a substantially increased risk of developing idiopathic venous thromboembolism (46, 47).

MTHFR POLYMORPHISM, GENETICS, AND ETHNICITY

The enzyme MTHFR catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor in the reaction converting homocysteine to methionine (48). Notably, at this metabolic locus, the reduced folates are directed either to protein and *S*-adenosylmethionine synthesis or to DNA and RNA synthesis (**Figure 2**). About 10% of most white populations are homozygous for a C to T transition at base pair 677. This polymorphism confers thermolability, reduced catalytic activity of the enzyme in vitro (48), and altered binding of the cofactor flavin adenine dinucleotide (49). Homozygous *TT* individuals are prone to elevated tHcy under conditions of impaired folate status (50), but the reduction in tHcy after folic acid supplementation is more pronounced in them than in those with the *CC* genotype (51).

In most populations investigated, those bearing the *TT* genotype have tHcy concentrations ≈25% higher than do those with the *CC* genotype. It has therefore been anticipated that the *TT* genotype confers increased CVD risk. However, meta-analyses including ≈6000 patients showed no significant relation between the C677T MTHFR polymorphism and CVD (10), or a borderline significant relation to the occurrence of coronary heart disease (52).

The fact that a major cause of hyperhomocysteinemia is not significantly associated with CVD has been taken as evidence that elevated tHcy is not a risk factor (13, 14). A key question is whether these arguments are tenable, as recently critically discussed by Fletcher and Kessling (53). If the risk of the *TT* genotype derives from its effect on tHcy, the expected relative risk can be computed from published data. In their meta-analysis, Brattström et al (10) found that the tHcy concentration was 2.6-μmol/L higher in those with the *TT* than in those with *CC* genotype. With use of data from prospective studies, a 5-μmol/L tHcy increment can be shown to be associated with an odds ratio (OR) of 1.20–1.30 (Figure 1). For a difference of 2.6 μmol/L, these ORs translate to 1.10 and 1.15, respectively. Standard sample size calculations show that to detect a relative risk in the range of 1.10–1.15 with a power of 80% and a significance level of 5%, 7800–16 300 cases and an equal number of controls are required (**Figure 3**; 10, 11, 54). This exceeds the sample size in any published study or meta-analysis of MTHFR and CVD (Figure 1). Thus, the nonsignificant relation between the C677T MTHFR polymorphism and CVD so far observed does not contradict the homocysteine theory. In fact, the relative risk of 1.12 associated

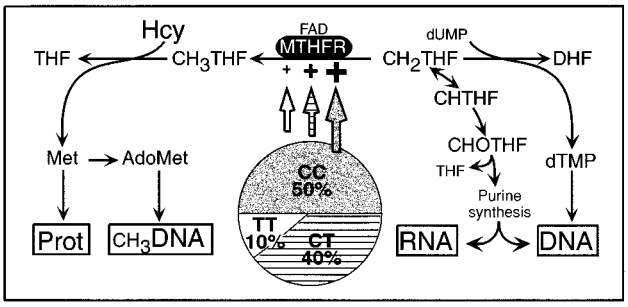

FIGURE 2. The C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism affects the distribution between folate species used for DNA and RNA syntheses and the 5-methyltetrahydrofolate form required for homocysteine remethylation and thereby protein synthesis. The pie chart in the center indicates the genotype prevalence often found in white populations and the associated vertical arrows the relation between genotype and MTHFR activity. AdoMet, *S*-adenosylmethionine; CH_3THF, 5-methyltetrahydrofolate; CH_2THF, 5,10-methylenetetrahydrofolate; CHTHF, methenyltetrahydrofolate; CHO, formyltetrahydrofolate; CH_3DNA, DNA methylation; DHF, dihydrofolate; Hcy, homocysteine; Met, methionine; Prot, protein; THF, tetrahydrofolate.

with the *TT* genotype that was reported by Brattström et al (10) agrees well with the expected relative risk calculated on the basis of our recent meta-analysis of prospective studies (Figure 1).

Another issue that has created some confusion is the large difference in the strength of association between the *TT* genotype and CVD in various studies. One reason may be marked differences in nutritional status of the various patient populations because *TT* individuals develop elevated tHcy only under conditions of impaired folate status (50, 55). In most clinical studies of MTHFR, folate and tHcy concentrations were not measured (53, 56), and in several reports showing no associations between C677T MTHFR and CVD risk, the authors stated that their study population was probably well nourished (53). In contrast, a recent study in Turkish men, who in general have a high prevalence of CVD, low cholesterol, and low folate, the *TT* genotype was a significant predictor of the extent of coronary artery disease (57).

Another source of erratic results is genetic heterogeneity of case compared with control populations. This has not been taken into account in many studies of MTHFR genotype and cardiovascular risk (53). Population specificity of allelic association has been thoroughly documented (53) and there are large interethnic variations in the frequency of the *T* allele, which varies from 0% in African blacks to 16% in Italians (53, 58, 59). Moreover, because of genetic or nutritional interactions, the C677T MTHFR polymorphism may predict CVD risk in only certain ethnic groups. In this context, it is notable that most studies of Japanese populations, comprising about 1400 patients and control subjects, showed a significant and occasionally strong association between the *TT* genotype and cardiovascular risk (60–65).

Because elevated tHcy concentration seems particularly harmful in subjects at high CVD risk, this may also be the case for the *TT* genotype. Some data support this possibility. In the largest study of the C677T MTHFR polymorphism and CVD risk undertaken to date, which included 2453 white male subjects, Gardemann et al (66) showed that the *TT* homozygosity was significantly associated with a graded coronary heart disease score obtained by angiography. Notably, this relation was confined to subjects with a high coronary risk as determined by a proatherogenic lipoprotein profile or elevated glucose concentration. Others have shown the synergistic effect of the MTHFR polymorphism and conventional risk factors in smaller studies (67). In a study of MTHFR and idiopathic venous thrombosis in an Israeli population, homozygosity for the MTHFR *T* allele was a risk factor showing a strong positive interactive effect with the prothrombotic polymorphisms factor V G1691A and prothrombin G20210A (68). Similar interactions were only occasionally shown in Italians (69, 70), but not in an English population (71), emphasizing the importance of ethnicity and genetic background.

Finally, the high prevalence of the C677T substitution of the MTHFR gene suggests that this genetic variant has certain advantages in connection with survival or reproduction that are probably related to folate intake and possibly riboflavin status. Thus, in our opinion, the *T* allele should not be regarded as a genetic defect, but rather as a trait that may affect disease susceptibility in both directions. In line with this, it has been shown that the *TT* homozygosity is associated with lower risk of colorectal cancer under conditions of low alcohol intake and positive folate status (72, 73). Moreover, it is conceivable that distribution of folates in the direction of purine and pyrimidine synthesis associated with the C677T MTHFR transition (Figure 2) protects against vascular disease by mechanisms independent of tHcy. This possibility recently gained some support. Demuth et al (74) found in asymptomatic subjects that elevated tHcy and the *TT* genotype were associated with opposite preclinical modifications of carotid artery geometry. This was explained by an enhanced eutrophic inward remodeling of the carotid artery in subjects with the *TT* genotype. Thus, the possibility that hyperhomocysteinemia and the *TT* genotype may have opposite effects on processes related to vascular occlusive disease may partly explain the inconsistent and weak relation of the MTHFR polymorphism with CVD (74).

FOLATE STATUS AND INTERVENTION WITH B VITAMINS

Several investigations (8), including prospective studies (75–78), showed that low intake or blood concentrations of folate confer increased CVD risk. Because folate status is the most important determinant of tHcy in the general population (79, 80), the associations between folate status and CVD support the concept of tHcy as a risk factor. However, a prothrombotic effect of impaired folate status independent of homocysteine (81) or lack of other protective micronutrients that are usually ingested together with folate cannot be excluded.

Currently, there has been no randomized, controlled trial of tHcy-lowering vitamins with hard clinical CVD endpoints (82). However, results from 3 intervention trials suggest that B vitamins have a protective effect. A combination of folic acid, vitamin B-6, and vitamin B-12 was reported to halt the rate of progression of carotid artery plaque area in 38 subjects with tHcy concentrations >14 μmol/L (83). In another study, 70 patients with post–methionine load hyperhomocysteinemia were given a combination of folate and vitamin B-6, and they had the same incidence rate of new cardiovascular events as did 162 patients with normal tHcy concentrations (84). A recent placebo-controlled trial of 158 healthy siblings of patients with premature atherothrombotic disease that used a combination of folic acid and vitamin B-6 showed a reduced occurrence of

abnormal exercise electrocardiographic tests and reduced fasting and post–methionine load tHcy concentrations in the treatment group (85). Because this vitamin combination reduces tHcy (85, 86), these preliminary findings are the first indications that tHcy-lowering therapy may protect against CVD, but an effect of vitamin B-6 independent of homocysteine (35, 87) cannot be ruled out.

PRECLINICAL VASCULAR DISEASE

Arterial intima-media wall thickness (IMT) is a measure of preclinical vascular disease, and it is associated with several conventional CVD risk factors (88). IMT is significantly related to tHcy in middle aged (89–91) and elderly (92) subjects. In a recent large study including 1111 subjects with a mean age of 52 y, the association between IMT and tHcy was of a strength similar to that of IMT and most traditional risk factors. The association between serum creatinine and IMT was weaker, which does not support the idea that impaired renal function is a confounder (91). Notably, a recent study showed that in chronic uremic hemodialysis patients, hyperhomocysteinemia is a predictor of IMT as strong as advanced age, systolic hypertension, and smoking (93).

The tHcy-IMT relation suggests that hyperhomocysteinemia precedes the acute CVD event and is present at an early stage of atherogenesis. This conclusion obtains strong support from the study of Tonstad et al (94), which reported that tHcy was related to IMT in both hypercholesterolemic and healthy children aged 10–19 y.

EVIDENCE FROM THE STUDY OF HOMOCYSTINURIA

The first and strongest evidence for elevated tHcy as a risk factor for atherothrombotic disease came from the study of homocystinuria. About 50% of untreated patients with cystathionine β-synthase deficiency have a major vascular occlusive event by the age of 30 y, despite the absence of traditional CVD risk factors. The fact that other forms of homocystinuria caused by different metabolic lesions such as MTHFR deficiency or various defects in cobalamin metabolism have a high occurrence of vascular disorders strengthens the case for elevated homocysteine as the causative agent. Different strategies, including pyridoxine, folic acid, cobalamin, or betaine supplementation, designed solely to lower tHcy concentration, have had a dramatic effect by nearly preventing the occurrence of vascular events. In 40 Australian patients with cystathionine β-synthase deficiency, there was an overall reduction in CVD events of 90% in 32 patients receiving tHcy-lowering therapy (95). In 25 Irish homocystinuria patients with 366 patient-years of treatment, no CVD event was recorded (96).

In 15 Australian (95) and 3 Irish (96) pyridoxine nonresponsive homocystinuria patients in whom plasma homocysteine concentrations remained substantially elevated (free homocysteine indicating tHcy in the range of 100 μmol/L) after therapy, no CVD event was recorded. Thus, even severe hyperhomocysteinemia may cause no cardiovascular event in the absence of other risk factors. This emphasizes the multifactorial genesis of vascular disease and points to the interactive character of hyperhomocysteinemia as a risk factor. In homocystinuric patients, such interactions have been reported with the factor V Leiden mutation in 3 consanguineous Israeli Arab families (97). This observation was not confirmed in cystathionine β-synthase–deficient patients recruited from France (98), the Netherlands (99), or Ireland (100), where other gene-gene interactions may prevail. The C677T MTHFR transition is an example of a genetic trait that may modify the effect of cystathionine β-synthase deficiency (99).

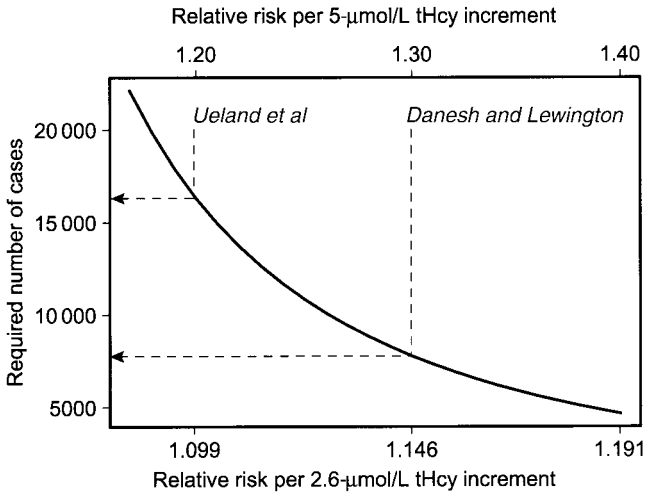

FIGURE 3. Assessment of the number of cases required in a case-control study with an equal number of cases and controls to detect the relative risk associated with the typical difference in total homocysteine (tHcy) of 2.6 μmol/L between individuals with *TT* and *CC* genotypes. This difference was obtained from the meta-analysis of methylenetetrahydrofolate reductase (MTHFR) polymorphism and cardiovascular disease by Brattström et al (10). The numbers obtained using the relative risk estimates from the updated meta-analyses of prospective studies given in this article and that of Danesh and Lewington (11) are illustrated by arrows. The sample size calculations were carried out with a power of 80%, a two-sided significance level of 5% (54), and by setting the *TT* genotype frequency among controls to 11.7% (10). Beresford is a member of the Ueland et al group (this article).

PLAUSIBLE MECHANISMS

Several mechanisms have been suggested for occlusive vascular disease associated with hyperhomocysteinemia. These involve platelets, the coagulation system, endothelium, and the vessel wall (101). Several mechanistic studies have been carried out with high homocysteine concentrations (1–10 mmol/L) never attained in vivo, and some effects obtained with homocysteine lacked specificity because they were also observed with other thiols (101).

Flow-mediated vasodilatation is a nitric oxide–mediated response observed after a transient brachial artery occlusion (102). This reactive mechanism is impaired in a dose-responsive manner in healthy subjects during the short-term hyperhomocysteinemia induced by methionine loading (103, 104). Furthermore, folic acid (105) and vitamin C (104) have a protective effect. Impaired flow-mediated vasodilatation is also observed in chronic hyperhomocysteinemic primates (101) and humans (106). In humans, both the elevated tHcy and the reactive vasodilatation are normalized after folic acid supplementation (107).

The rapid impairment of flow-mediated vasodilatation associated with increased tHcy concentration in vivo lends strong support to the idea that elevated homocysteine provokes an acute vascular event, particularly in subjects with other CVD risk factors. The fact that the response is observed in healthy subjects precludes confounding by other risk factors. A mechanism involving nitric oxide–dependent endothelial function may account for the arterial and venous occlusions associated with

moderate to severe hyperhomocysteinemia, including homocystinuria. Finally, impaired flow-mediated vasodilatation is associated with numerous other CVD risk factors (108), including aging (109), hypertension (110), hypercholesterolemia (110), smoking (111), and diabetes (112), and these associations are in accordance with the enhanced effect of hyperhomocysteinemia in the presence of conventional CVD risk factors (108).

The results of some in vivo experiments in humans add further credence to the concept that elevated tHcy may provoke an acute vascular lesion. The acute hyperhomocysteinemia after methionine loading is associated with acute endothelemia (113), an increase in soluble adhesion molecules, increments of several coagulation variables, and impaired hemodynamic and rheologic responses to L-arginine (114).

HYPERHOMOCYSTEINEMIA AS AN EPIPHENOMENON

The observation that the *TT* MTHFR genotype is associated with no or only a minor enhancement of CVD risk (14) is not a valid argument against the homocysteine theory, as outlined above. The fact that tHcy is related to a diverse array of established risk factors, including age, sex, smoking, exercise, impaired renal function, and blood pressure (79, 115), could suggest that the association between tHcy and CVD is due to confounding. The alternative explanation is that the high tHcy concentration partly mediates the risk associated with some of these factors. If the latter is the case, assessment of the CVD risk associated with hyperhomocysteinemia after adjustment for these potential confounders may actually lead to risk underestimation. Notably, most studies suggested that tHcy is independent of and even enhances the risk associated with the conventional risk factors, such as smoking, hypertension, hypercholesterolemia, diabetes, and renal failure (6, 8).

From the close relation between plasma tHcy and renal function (18, 116), it has been inferred that vascular disease may cause hyperhomocysteinemia by impairment of renal function. However, there are ≥ 4 prospective studies that consistently showed that elevated tHcy is a strong predictor of CVD in patients with end-stage renal failure and in renal transplant recipients, suggesting that hyperhomocysteinemia is not merely a benign epiphenomenon of renal dysfunction (117, 118).

It has been argued that tHcy increases secondary to the myocardial or cerebrovascular event. This assumption is based on the observations of low tHcy in the acute phase (first days) after myocardial infarction or stroke compared with the convalescent stage (119–121). An alternative explanation is a transient drop in tHcy during the acute phase, which would weaken rather than strengthen the tHcy-CVD association. Furthermore, an altered tHcy concentration after the CVD event does not affect the interpretation of the prospective data.

CONCLUSION

The case of homocystinuria, the results of most prospective studies, and the relation between hyperhomocysteinemia and preclinical atherosclerosis suggest that elevated tHcy is a causal risk factor for CVD, including venous thrombosis. Hyperhomocysteinemia as an isolated phenomenon probably confers minor risk, but it further increases the risk when it occurs in combination with other factors that provoke vascular lesions. Thus, hyperhomocysteinemia seems to be a particularly strong risk factor in subjects with an underlying disease and predicts the short-term outcome in such individuals. The impairment of the nitric oxide–dependent flow-mediated vasodilatation during transient hyperhomocysteinemia provides one plausible mechanism accounting for the acute effect. Finally, lack of a significant association between the C677T MTHFR polymorphism and CVD does not take way from the concept of homocysteine as a risk factor because published studies lack the power to detect the risk enhancement associated with the moderate elevation of tHcy detected in subjects with the *TT* genotype. In addition, this genetic variant has a profound effect on overall intracellular folate distribution, which may modulate or even reduce CVD risk.

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