Homocyst(e)ine and Cardiovascular Disease: A Critical Review of the Epidemiologic Evidence

John W. Eikelboom, MBBS, FRACP; Eva Lonn, MD, FRCPC; Jacques Genest Jr., MD, FRCPC; Graeme Hankey, MBBS, MD, FRCP, FRCP(Edin), FRACP; and Salim Yusuf, MBBS, DPhil, FRCPC

Purpose: To review epidemiologic studies on the association between homocyst(e)ine level and risk for cardiovascular disease and the potential benefits of homocysteine-decreasing therapies.

Data Sources: Computerized and manual searches of the literature on total homocysteine levels and cardiovascular disease.

Study Selection: Prospective studies and major retrospective epidemiologic studies evaluating the association between homocyst(e)ine levels and cardiovascular disease and the association between blood levels or dietary intake of folate, vitamin B_6, and vitamin B_{12} and cardiovascular disease.

Data Extraction: Relevant data on patient population, plasma homocyst(e)ine levels, duration of follow-up, and main results were extracted from studies that met the inclusion criteria.

Data Synthesis: The designs and results of studies included in this review are summarized. A formal meta-analysis was not performed because the studies were heterogeneous in method and design.

Conclusions: Results of epidemiologic studies suggest that moderately elevated plasma or serum homocyst(e)ine levels are prevalent in the general population and are associated with an increased risk for cardiovascular disease, independent of classic cardiovascular risk factors. Simple, inexpensive, nontoxic therapy with folic acid, vitamin B_6, and vitamin B_{12} reduces plasma homocyst(e)ine levels. Although the association between homocyst(e)ine levels and cardiovascular disease is generally strong and biologically plausible, the data from the prospective studies are less consistent. In addition, epidemiologic observations of an association between hyperhomocyst(e)inemia and cardiovascular risk do not prove the existence of a causal relation. Therefore, the effectiveness of folate, vitamin B_6, and vitamin B_{12} in reducing cardiovascular morbidity and mortality requires rigorous testing in randomized clinical trials. Several such trials are under way; their results may greatly affect cardiovascular morbidity and mortality, given the simplicity and low cost of vitamin therapy.

Cardiovascular disease remains the major cause of morbidity and death in developed countries and accounts for approximately 40% of all deaths in Canada (1). Smoking cessation and reductions in cholesterol levels and blood pressure have been shown to be effective strategies in the prevention of cardiovascular disease (2). However, these major, classic cardiovascular risk factors and such nonmodifiable risk factors as age, sex, and family history cannot fully explain why some persons develop myocardial infarction, stroke, and other cardiovascular disease but other persons do not (3–5). Other factors may also increase the likelihood of developing cardiovascular disease and contribute to atherogenesis. Pathologic and epidemiologic studies suggest that only about one half to two thirds of the variation in anatomic extent of atherosclerosis and risk for atherosclerotic vascular disease can be explained by classic risk factors (6–9). Therefore, many emerging risk factors have been investigated. Among these, elevated plasma or serum levels of homocyst(e)ine (hyperhomocyst(e)inemia) are of particular interest. Recent epidemiologic studies have shown that moderately elevated plasma homocysteine levels are highly prevalent in the general population and are associated with an increased risk for fatal and nonfatal cardiovascular disease, independent of classic cardiovascular risk factors. This association is usually consistent, strong, dose-related, and biologically plausible. Although simple, inexpensive, nontoxic therapy with folate and vitamins B_6 and B_{12} is highly effective at reducing plasma homocyst(e)ine levels, it remains to be demonstrated that decreasing homocyst(e)ine levels reduces cardiovascular morbidity and mortality.

Methods

Data Sources

We searched the scientific literature for all epidemiologic studies (prospective, case–control, cross-

Ann Intern Med. 1999;131:363-375.

For author affiliations and current addresses, see end of text.

See editorial comment on pp 387-388.

sectional, or geographic correlation) on cardiovascular disease (using the terms *coronary heart disease*, *cerebrovascular disease*, *peripheral vascular disease*, and *atherosclerosis*) and homocysteine or vitamins (using the terms *homocysteine*, B_{12}, B_6, and *folic acid*). We searched the MEDLINE database for articles published from January 1965 to January 1999 and identified additional studies by examining bibliographies of original articles, review articles, and textbooks.

Study Selection

We used standard definitions to define epidemiologic studies (10) and did not consider case series. The epidemiologic prospective cohort studies varied greatly in terms of patient selection, number of patients and controls, circumstances and techniques of measuring plasma homocyst(e)ine levels, definitions of elevated plasma homocyst(e)ine level, types and definitions of vascular outcome events and surrogate outcome measures, and statistical analyses. A formal meta-analysis of these studies was not performed because the results could have been misleading. We included all prospective epidemiologic studies (up to January 1999) but included only the largest retrospective studies (those that involved at least 150 cases) because prospective studies generally provide a robust estimate of association, whereas small retrospective studies are often subject to various biases. We further restricted the retrospective studies to those published after the meta-analysis by Boushey and colleagues (11) because that article provides a good critical review of the studies done up until 1995.

Homocysteine Metabolism

Homocysteine is a sulfur-containing amino acid produced during catabolism of the essential amino acid methionine. Homocysteine can be metabolized by two major pathways. When methionine is in excess, homocysteine is directed to the transsulphuration pathway, where it is irreversibly sulfoconjugated to serine by cystathionine β-synthase in a process requiring vitamin B_6 as a cofactor. However, under conditions of negative methionine balance, homocysteine is primarily metabolized through a methionine-conserving remethylation pathway. In most tissues, homocysteine is remethylated in a process that requires methionine synthase, vitamin B_{12} as a cofactor, and methyltetrahydrofolate as a cosubstrate. This pathway requires an adequate supply of folic acid and the enzyme methylene tetrahydrofolate reductase (MTHFR) (12). Genetic and acquired abnormalities in the function of these enzymes or deficiencies in folic acid, vitamin B_6, or vitamin B_{12} cofactors can lead to elevated homocysteine levels.

In the plasma, approximately 70% of homocysteine circulates in a protein-bound form; approximately 25% combines with itself to form the dimer homocystine; and the remainder (<5%) combines with other thiols, including cysteine, to form disulphide (a homocysteine–cysteine mix) or circulates as the free thiol compound (13). In North America, the term *homocyst(e)ine* is often used to refer to the total pool of circulating plasma homocysteine, whereas the term *tHcy* is more common in Europe.

Homocyst(e)ine Theory of Atherosclerosis

Severe hyperhomocyst(e)inemia associated with homocystinuria can be caused by several rare inherited disorders, including homozygous deficiency of cystathionine β-synthase, MTHFR, or methionine synthase or defects in vitamin B_{12} metabolism (12, 14). These distinct genetic conditions share the following features: extreme elevations of plasma homocyst(e)ine levels and premature atherothrombotic disease with typical histopathologic features of endothelial injury, proliferation of vascular smooth-muscle cells, progressive arterial stenosis, and hemostatic changes suggestive of a prothrombotic state (15). The characteristic clinical and pathologic features of these genetically diverse conditions support the hypothesis that elevated plasma homocyst(e)ine levels are responsible for the vascular damage and led McCully and Wilson (16) to propose the "homocyst(e)ine theory of atherosclerosis." Although these genetic errors of metabolism are extremely rare (homozygous cystathionine β-synthase deficiency occurs in approximately 1 in 150 000 live births and is associated with plasma homocyst(e)ine levels as high as 400 μmol/L), they provide a useful in vivo human model for vascular injury associated with high homocyst(e)ine levels.

Laboratory Measurement of Homocyst(e)ine Levels

Most assays for measuring homocysteine concentrations are based on chromatographic techniques; high-performance liquid chromatography is still the most widely used (13). However, a simple and relatively inexpensive immunoassay has become commercially available and may soon enable widespread measurement of plasma homocyst(e)ine levels in the clinical laboratory (17). Plasma homocyst(e)ine levels are usually measured in the fasting state and can be measured before or after methionine loading.

Methionine loading, a method of stressing the homocyst(e)ine metabolic pathways, may be more sensitive than measurement of fasting homocyst(e)ine levels for detecting mild disturbances in the transsulfuration pathway that may be caused by vitamin B_6 deficiency or partial cystathionine β-synthase deficiency (18, 19). The procedure involves measuring the baseline fasting plasma homocyst(e)ine level, administering a standard oral dose of methionine, and measuring the plasma homocyst(e)ine level again 4 to 6 hours later. Methionine loading may help to discriminate between defects involving the transsulfuration and remethylation pathways (20); it may also help to identify patients who have impaired homocysteine metabolism despite a normal fasting total plasma homocysteine level and who may, therefore, be at increased risk for vascular disease (19).

Reliable measurements of plasma homocyst(e)ine levels require the use of accurate assays as well as optimal procedures for collection and handling of blood samples (13). Patient characteristics (such as fasting state and posture) and recent vascular events may also affect measured total homocysteine levels (20–22).

Table 1. Causes of Hyperhomocyst(e)inemia

Enzyme deficiencies and mutations
Cystathionine β-synthase
Methionine synthase
Methylenetetrahydrofolate reductase
Cobalamin mutations
Vitamin deficiencies
Folate
Vitamin B_6
Vitamin B_{12}
Increased methionine consumption
Demographic characteristics
Increasing age
Male sex
Tobacco use
Physical inactivity
Postmenopausal status
Chronic medical disorders
Decreased renal function
Systemic lupus erythematosus
Malignant neoplasms
Hyperproliferative disorders
Severe psoriasis
Hypothyroidism
Diabetes mellitus
Transplantation
Acute-phase response to illness
Drugs
Anticonvulsant agents (phenytoin, carbamazepine)
Folate antagonists (methotrexate)
Vitamin B_{12} antagonists (nitrous oxide)
Vitamin B_6 antagonists
Cholesterol-lowering agents (cholestyramine, colestipol, nicotinic acid)
Thiazide diuretics
Cyclosporine

Definition and Prevalence of Hyperhomocyst(e)inemia

Hyperhomocyst(e)inemia is usually defined by using arbitrary cut-off points—for example, above the 95th percentile or more than two SDs above the mean of values obtained from fasting, healthy controls. This is similar to the way in which high plasma cholesterol levels were originally defined. Normal plasma homocyst(e)ine levels usually range from 5 to 15 μmol/L (17). However, the definition of elevated homocyst(e)ine levels is not standardized, and substantial differences exist in the "normal" reference levels used in the literature. Higher fasting values are arbitrarily classified as mild and moderate hyperhomocyst(e)inemia (16 to 100 μmol/L) and severe hyperhomocyst(e)inemia (>100 μmol/L). The prevalence of hyperhomocyst(e)inemia depends on the way in which the condition is defined and measured. When the common definition of hyperhomocysteinemia—levels of total homocysteine exceeding the 95th percentile of the distribution in a healthy sample of controls—is used, 5% of the normal population will necessarily be defined as having an elevated homocyst(e)ine level (23). Between 13% and 47% of patients with symptomatic atherosclerotic vascular disease have been reported to have hyperhomocystein(e)mia (24). However, little evidence suggests a sudden increase in risk for vascular disease above a certain threshold level of plasma homocyst(e)ine; the relation between plasma homocyst(e)ine levels and risk for cardiovascular disease seems to be graded and linear (25).

Causes of Mild and Moderate Hyperhomocyst(e)inemia

One or a combination of genetic, physiologic, pathologic, and nutritional factors (**Table 1**) causes modest elevations in homocyst(e)ine levels without associated homocystinuria. Therefore, *MTHFR* mutations (for example, thermolabile *MTHFR*); older age; male sex; postmenopausal status; smoking; sedentary lifestyle; dietary factors, including increased intake of animal proteins (which have a higher methionine content); low intake of folic acid, vitamin B_6, and vitamin B_{12}; decreased renal function; transplantation; and such medications as corticosteroids and cyclosporine have been associated with mild and moderate hyperhomocysteinemia (26, 27).

Of particular interest are nutritional deficiencies in the vitamin cofactors that are required for homocysteine metabolism: folic acid, vitamin B_6, and vitamin B_{12}. These deficiencies are highly prevalent and may account for most cases of moderate hyperhomocysteinemia. An inverse relation has been shown between plasma homocyst(e)ine levels and plasma levels and dietary intake of folate and vita-

Table 2. Hyperhomocyst(e)inemia and Anatomic Extent of Atherosclerosis*

Study (Reference)	Design	Selection Criteria	Participants
Malinow et al. (56)	Case–control	Population sample (ARIC cohort)	287 case-patients, 287 controls
Aronow et al. (57)	Case–control	Not described	121 men, 79 women
Tonstad et al. (58)	Case–control	Children with family history; normal controls	90 case-patients, 30 controls
Clarke et al. (59)	Case–control	Cystathionine β-synthase–deficient heterozygotes; normal controls	25 case-patients, 21 controls
Selhub et al. (60)	Cross-sectional	Population sample (Framingham cohort)	1041 adults
Verhoef et al. (61)	Case–control	CAD on angiography; controls had no CVD	131 case-patients, 101 controls
Montalescot et al. (62)	Case–control	Symptomatic CAD, CVD, or PVD; normal controls	75 case-patients, 75 controls
van den Berg et al. (63)	Cross-sectional	Symptomatic PVD	171 case-patients
Konecky et al. (64)	Cross-sectional	Patients referred for tranesophageal echocardiography	156 adults

* ARIC = Atherosclerosis Risk in Communities Study; CAD = coronary artery disease; CVD = cardiovascular disease; OR = odds ratio; PVD = peripheral vascular disease.
† Highest compared with lowest fifths of plasma homocyst(e)ine level.
‡ Per unit increase in homocyst(e)ine level.
§ Highest compared with lowest quarters of homocyst(e)ine level.
‖ For each 5-μmol/L increase in homocyst(e)ine level.
¶ In patients with coronary disease (n = 50) and matched controls (n = 50).
** Upper quarter compared with lower three quarters.

mins B_6 and B_{12} (26), and fasting homocyst(e)ine levels are elevated in more than 95% of patients with folic acid or vitamin B_{12} deficiency (27). Vitamin B_6 deficiency is associated primarily with hyperhomocyst(e)inemia after methionine loading. Selhub and coworkers (26) reported that low plasma levels of folate or B vitamins were present in about two thirds of all patients with hyperhomocyst(e)inemia and that up to 40% to 50% of a North American sample of elderly persons consume insufficient amounts of folic acid and vitamin B_6 (26). In the United States, 80% to 90% of the population has a dietary folate intake less than 400 μg/d (the new suggested recommended daily allowance); national averages for folate intake and vitamin B_6 are estimated at 224 μg/d and 1.51 mg/d, respectively (28, 29). in Europe, the mean dietary folate intake is reported to be 291 μg/d (range, 197 to 326 μg/d) for men and 247 μg/d (range, 168 to 320 μg/d) for women. The desired intake was reached by only a small proportion of the European persons studied (30).

Total Homocysteine Level and Atherogenesis: Experimental Evidence and Possible Mechanisms

Experimental evidence suggests that the atherogenic propensity associated with hyperhomocyst(e)inmia is caused by endothelial dysfunction and injury, which in turn is followed by platelet activation and thrombus formation. Numerous mechanisms have been suggested by which hyperhomocysteinemia may contribute to atherothrombotic vascular disease, including direct toxic endothelial cell damage. Direct toxic endothelial cell damage has been shown in both in vitro and in vivo models (including experimental evidence in primates) to be related primarily to 1) the generation of potent reactive oxygen species (31–35); 2) impaired production of endothelium-derived nitric oxide and endothelial dysfunction, as evidenced by impaired endothelial-dependent vascular reactivity in primates and in humans with elevated homocyst(e)ine levels (36–39); 3) stimulation of smooth-muscle cell proliferation (40, 41); and 4) lipid abnormalities, including elevated plasma triglyceride levels and increased susceptibility to oxidation of low-density lipoproteins (42–44). Another suggested mechanism is increased thrombogenicity mediated by increased platelet adherence and the release of platelet-derived growth factors secondary to homocyst(e)ine-induced endothelial damage; activation of factors V, X, and XII; inhibition of protein C activation; inhibition of cell surface expression of thrombomodulin; and decreased tissue plasminogen activator activity (45–50). A potentially unifying hypothesis of the vascular damage associated with hyperhomocysteinemia relates to the formation of oxygen free radicals, which cause oxidative vascular damage, proliferation of smooth-muscle cells, alteration in endothelial function and structure, and increased thrombogenicity that ultimately leads to atherothrombosis (51).

Table 2—*Continued*

Age Range	Major Outcomes	Mean Plasma Homocyst(e)ine Level		Main Results
		Case-Patients	Controls	
y		*μmol/L*		
	Carotid intima–media thickness	9.26	8.32	OR, 3.15 (CI, 1.57–6.32)†
60–99	Extracranial carotid stenosis (case-patients: 40%–100%; controls: 0%–39%)	Men: 19; women: 17	Men: 14; women: 13	$P = 0.003$ for men; $P < 0.001$ for women (difference in plasma homocyst(e)ine level between case-patients and controls)
10–19	Carotid intima–media thickness	Boys: 6.7; girls: 6.0	Boys: 6.3, girls: 6.3	$r = 0.22$ (correlation between plasma homocyst(e)ine level and intima–media thickness)
	Carotid intima–media thickness	56.0	16.0	OR, 1.04 (CI, 1.0–1.07)‡
67–96	Extracranial carotid stenosis ≥25%	–	–	OR, 2.0 (CI, 1.4–2.9)§
25–65	Angiographic CAD	13.5	12.5	OR, 1.3 (CI, 1.0–1.6)‖
	Angiographic CAD (number of vessels)	11.7§	9.9¶	$r = 0.25$ (correlation between plasma homocyst(e)ine level and extent of coronary atherosclerosis)
<55	Angiographic PVD	–	–	OR, 4.0 (CI, 1.6–9.9)**
30–89	Aortic plaque surface area	–	–	$r = 0.63$ (correlation between plasma homocyst(e)ine level and extent of aortic atherosclerosis)

Epidemiologic Associations between Hyperhomocyst(e)inemia and Cardiovascular Risk

Genetic Studies

A highly prevalent C677T point mutation of the *MTHFR* gene is associated with a thermolabile *MTHFR* variant. This mutation has been reported in 38% of French Canadians; the *TT* genotype is found in 5% to 15% of the general population in Canada and is associated with elevated plasma homocyst(e)ine levels, particularly in the setting of reduced folate levels (52–54). Frosst and colleagues (52) suggested that the C677T polymorphism in the *MTHFR* gene was a candidate risk factor for vascular disease. However, most individual studies and a recent meta-analysis of studies involving more than 6000 genotyped patients and controls have failed to confirm this association (55). This apparent paradox—that the *TT* genotype in the *MTHFR* gene, which is associated with high homocyst(e)ine levels and is a strong predictor for hyperhomocystein(e)mia in the general population, is not consistently associated with increased cardiovascular risk—remains unclear. Potential explanations include the relatively low frequency of the TT genotype, the association of the TT genotype with elevated homocyst(e)ine levels only in the presence of folate deficiency, and methodologic limitations of available data (such as the relatively small sample size of most studies).

Studies of the Association between Plasma Homocyst(e)ine Levels and Anatomic Extent of Atherosclerosis

Cross-sectional and case–control studies have shown a clear association between plasma homocyst(e)ine levels and the anatomic extent of carotid (56–60), coronary (61, 62), peripheral vascular (63), and aortic (64) atherosclerotic disease (**Table 2**). These variables are, however, only surrogate measures of cardiovascular events.

Cross-Sectional and Retrospective Case–Control Studies

Many cross-sectional and retrospective observational studies have examined the association between plasma homocyst(e)ine level and cardiovascular risk, and most support the existence of such an association. Despite the enormous heterogeneity of the studies, a 1995 meta-analysis of 27 observational studies involving a total of approximately 4000 participants reported that hyperhomocyst(e)inemia (defined as plasma homocyst(e)ine levels above the 90th or 95th percentile of levels in controls) was associated with an increased risk for fatal and nonfatal atherosclerotic vascular disease in the coronary (odds ratio, 1.7 [95% CI, 1.5 to 1.9]), cerebral (odds ratio, 2.5 [CI, 2.0 to 3.0]), and peripheral (odds ratio, 6.8 [CI, 2.9 to 15.8]) circulation (11). An increase of 5 μmol/L in basal total plasma homocysteine level was associated with a 60% increase (CI, 40% to 70%) in the odds of coronary heart disease among men and an 80% increase (CI, 30%

to 90%) in the odds of coronary heart disease among women; it was also associated with a 50% increase (CI, 30% to 90%) in the odds of cerebrovascular disease. On the basis of this report, it was estimated that a reduction of 5 μmol/L in plasma homocyst(e)ine level would decrease vascular risk by one third. However, these studies (which measured homocyst(e)ine levels only at baseline) may have underestimated the strength of the association. After correction for the "regression dilution bias," a prolonged lower total plasma homocysteine level of approximately 3 to 4 μmol/L corresponds to approximately one-third less vascular disease. An increase of 5 μmol/L was estimated to increase the risk for coronary heart disease by as much as does an increase in cholesterol level of 0.5 mmol/L (19 mg/dL). This meta-analysis also estimated that approximately 10% of the coronary heart disease in the general population could be attributed to hyperhomocyst(e)inemia.

Since publication of this meta-analysis, many additional observational studies have been done (19, 65–71). Most of these studies have also reported an association between hyperhomocyst(e)inemia and atherosclerotic vascular disease (**Table 3**). The recent large European Concerted Action Project (19), which involved 750 patients with arterial vascular disease and 800 controls, confirmed that an elevated plasma homocyst(e)ine level was an independent risk factor for cardiovascular disease (odds ratio, 2.2 [CI, 1.6 to 2.9]) and calculated that an increase of 5 μmol/L in fasting basal homocyst(e)ine level was associated with a relative risk for cardiovascular disease of 1.35 (CI, 1.1 to 1.6) in men and 1.42 (CI, 0.99 to 2.55) in women.

Prospective Cohort Studies

Prospective longitudinal studies provide more robust evidence, especially when a strong and consistent association between a risk factor and disease is found (although even these investigations cannot fully account for possible confounders). However, the results of prospective cohort studies that evaluated the association between hyperhomocyst(e)inemia and vascular risk remain inconclusive (**Table 4**). Eight cohort studies reported statistically significant positive associations between elevated homocyst(e)ine levels and cardiovascular disease. A large prospective cohort study from Tromsø, Norway (25), reported a relative risk for coronary heart disease of 1.41 (CI, 1.16 to 1.71) for each increase of 4 μmol/L in serum homocyst(e)ine level; there was no threshold level below which homocyst(e)ine was not associated with risk for coronary heart disease. After 5 years of follow-up, the Physicians' Health Study (72) found an adjusted relative risk for fatal or nonfatal myocardial infarction of 3.4 (CI, 1.3 to 8.8; $P = 0.01$) for persons whose homocyst(e)ine levels were in the highest 5% compared with those whose homocyst(e)ine levels were in the lowest 90%. The British United Provident Association study (73) reported a risk for fatal coronary heart disease of 2.9 (CI, 2.04 to 4.12) among men whose homocyst(e)ine level was in the highest quarter compared with those whose homocyst(e)ine level was in the lowest quarter, after adjustment for other

Table 3. Recent Cross-Sectional and Retrospective Case–Control Studies of Homocysteine and Cardiovascular Risk*

Study (Reference)	Design	Patient Selection	Setting	Participants
Graham et al. (19)	Case–control	Men and women with vascular disease	Nine European countries	750 case-patients, 800 controls
Markus et al. (65)	Case–control	Men and women with ischemic stroke	United Kingdom	160 case-patients, 75 controls
Lindgren et al. (66)	Case–control	Consecutive men and women with acute stroke	Sweden	162 case-patients, 79 controls
Hopkins et al. (67)	Case–control	Men and women with CAD and family history of CAD	United States	304 case-patients, 231 controls
Dalery et al. (68)	Case–control	Men and women with angiographic CAD	Quebec, Canada	420 case-patients, 521 controls
Robinson et al. (69)	Case–control	Men and women with angiographic CAD	United States	162 case-patients, 155 controls
Malinow et al. (70)	Case–control	Men with previous myocardial infarction	Ireland and France	150 case-patients, 584 controls
Alfthan et al. (71)	Cross-sectional, ecological	Healthy men	European countries	1990 men

* All studies were published after publication of the meta-analysis by Boushey et al. (11). CAD = coronary artery disease; CVD = cardiovascular disease; NS = not significant; OR = odds ratio.
† Highest compared with lower fifths of total homocysteine levels.
‡ Reported as log total homocysteine.
§ Highest compared witih lowest fifths of total homocysteine levels.

risk factors. The British Regional Heart Study (74) found an independent, graded, positive association between hyperhomocyst(e)inemia and the risk for stroke. Nygård and coworkers (75) reported a strong graded relation between plasma homocyst(e)ine levels and overall mortality in persons with angiographically demonstrated coronary heart disease; the mortality ratio was 4.5 (CI, 1.22 to 16.6) for persons with the highest levels of homocyst(e)ine compared with those with the lowest levels. A smaller prospective study from Zutphen, the Netherlands (76), reported a positive association between homocyst(e)ine levels and risk for myocardial infarction and stroke; a prospective study in patients with systemic lupus erythematosus found a positive association between homocyst(e)ine levels and risk for atherothrombotic events (77); and the Rotterdam Study (78) reported odds ratios of 2.53 (CI, 1.19 to 5.35) for stroke and 2.43 (CI, 1.11 to 5.35) for myocardial infarction in persons whose plasma homocyst(e)ine level was in the highest fifth.

However, the above results were not confirmed in six reports from four large cohorts followed prospectively. A more recent report from the Physicians' Health Study (79) failed to demonstrate a significant association between plasma homocyst(e)ine levels and risk for myocardial infarction or death from coronary heart disease (relative risk, 1.7 [CI, 0.9 to 3.3]) after a more prolonged follow-up of 7.5 years; this cohort also showed no significant associations between plasma homocyst(e)ine level and risk for stroke and angina (80, 81). Similarly, a large study from Kuopio, Finland (82); the Multiple Risk Factor Intervention Trial cohort (83); and the recent report on the Atherosclerosis Risk in Communities Study cohort (84) did not find significant associations between hyperhomocyst(e)inemia and fatal and nonfatal coronary heart disease and stroke. Four of these studies (79, 80, 82, 84) showed a trend toward increased risk associated with high levels of homocyst(e)ine, and it is possible that the use of less extreme contrasts (for example, comparison of event rates in patients whose plasma homocyst(e)ine level was in the upper 5% with rates in patients whose plasma homocyst(e)ine level was in the lower 95%) contributed to the lack of statistically signficant results.

Association between Dietary Intake and Blood Levels of Folate, Vitamin B_6, and Vitamin B_{12} and Cardiovascular Risk

Most cross-sectional, retrospective, case–control, and prospective cohort studies show positive and graded (dose-dependent) associations between blood levels or dietary intake of folate and vitamins B_6 and B_{12} and risk for cardiovascular disease; however, the epidemiologic data are not entirely consistent. The largest studies (30, 80, 85–89) are summarized in **Table 5**.

Epidemiologic studies cannot fully exclude the possibility that folate and vitamins B_6 and B_{12} may have an association with cardiovascular risk and atherogenesis that is independent of homocyst(e)ine levels or that a high level of these vitamins may be a marker of diets rich in fruits and vegetables, which have been independently associated with

Table 3—*Continued*

Age	Total Plasma Homocysteine Level		Main Results
	Case-Patients	Controls	
y	*μmol/L*		
mean ± SD, 47.2 ± 0.3	11.3	9.7	Relative risk, 2.2 (CI, 1.6–2.9)†
mean, 65	1.32‡	1.27‡	$P = 0.09$ (difference in log homocyst(e)ine level between patients and controls)
≥50	13.4	13.8	P = NS (difference in homocyst(e)ine level between case-patients and controls)
mean ± SD, 62 ± 11	Men: 13.7; women: 12.6	Men: 11.3; women: 8.9	Men: OR, 13.8 (CI, 3.5–55); women: OR, 12.8 (CI, 2.0–82) for homocyst(e)ine level >19 μmol/L compared with ≤9 μmol/L
25–64	Men: 11.7, women: 12.0	Men: 9.7; women: 7.6	Men: $P < 0.001$; women: $P < 0.01$ (difference in homocyst(e)ine level between case-patients and controls)
38–68	Men: 13.9; women: 15.3	Men: 11.2; women: 10.1	Men: OR, 0.29 (CI, 1.7–4.7); women: OR, 3.5 (CI, 1.4–8.5)†
20–59	Ireland: 15.5; France: 16.7	Ireland: 14.7; France: 12.9	Ireland: OR, 3.42 (CI, 1.6–7.2); France: OR, 5.18 (CI, 2.9–9.3)§
40–49	Elevated total homocysteine levels were found in countries with higher CVD mortality rate		$r = 0.71$ (correlation between homocyst(e)ine level and CVD death)

Table 4. Prospective Cohort Studies of Homocysteine and Risk for Cardiovascular Disease*

Study (Reference)	Design	Patient Selection	Setting	Participants
Stampfer et al. [Physicians' Health Study] (72)	Nested case–control	14 916 male physicians	United States	271 case-patients, 271 controls
Wald et al. [British United Provident Association Study] (73)	Nested case–control	21 520 men	United Kingdom	229 case-patients, 1126 controls
Arneson et al. (25)	Nested case–control	10 963 men, 10 863 women	Tromsø, Norway	123 case-patients, 492 controls
Perry et al. [British Regional Heart Study] (74)	Nested case–control	5661 men	United Kingdom	107 case-patients, 118 controls
Nygård et al. (75)	Nested case–control	587 men and women with angiographic CAD	Belgium	64 case-patients
Stehouwer et al. [Zutphen Study] (76)	Nested case–control	878 men	The Netherlands	162 case-patients
Petri et al. (77)	Prospective cohort	337 patients with systemic lupus erythematosus	United States	60 case-patients
Bots et al. [Rotterdam Study] (78)	Nested case–control	7983 men and women	The Netherlands	224 case-patients, 533 controls
Chasan-Taber et al. [Physicians' Health Study] (79)	Nested case–control	14 916 male physicians	United States	333 case-patients, 333 controls
Verhoef et al. [Physicians' Health Study] (80)	Nested case–control	14 916 male physicians	United States	109 case-patients, 427 controls
Verhoef et al. [Physicians' Health Study] (81)	Nested case–control	14 916 male physicians	United States	149 case-patients, 149 controls
Alfthan et al. [North Karelia Project] (82)	Nested case–control	7424 men and women	Finland	265 case-patients, 269 controls
Evans et al. [Multiple Risk Factor Intervention Trial] (83)	Nested case–control	12 866 men	United States	93 patients with MI, 186 controls; 147 patients who died of CHD, 286 controls
Folsom et al. [Atherosclerosis Risk in Communities Study] (84)	Nested case–control	15 792 men and women	United States	232 case-patients, 537 controls

* CAD = coronary artery disease; CABG = coronary artery bypass graft surgery; CHD = coronary heart disease; MI = myocardial infarction.
† For ≥95th percentile compared with ≤10th percentile of total homocysteine levels.
‡ Highest compared with lowest quartiles of total homocysteine levels.
§ Per 4-μmol/L increment in homocysteine level.
‖ Homocyst(e)ine levels ≥20 μmol/L compared with those <9 μmol/L.
¶ Highest compared with lowest third of total homocysteine levels.
** Highest compared with lowest fifth of total homocysteine levels.
†† ≥95th percentile compared with <95th percentile of total homocysteine levels.
‡‡ ≥95th percentile compared with ≤75th percentile of total homocysteine levels.

lower risk for cardiovascular disease and may contain other vascular protective substances (90). Despite some inconsistencies in the studies of vitamins and the inherent weaknesses of epidemiologic investigations, these associations strengthen the validity of the "homocyst(e)ine theory of atherosclerosis."

Summary of Epidemiologic Data

Emerging evidence from epidemiologic studies supports a strong, dose-dependent, positive association between plasma homocyst(e)ine level and risk for cardiovascular disease. This association seems to be independent of other known risk factors associated with elevated total homocysteine levels and other cardiovascular risk factors. However, the conclusion that an elevated plasma homocyst(e)ine level is an independent risk factor for atherothrombotic vascular disease should be tempered by the inconsistent findings of prospective cohort studies, the failure to demonstrate consistent associations between genetic mutations that cause elevated plasma homocyst(e)ine levels and cardiovascular risk, and the absence of data from randomized clinical trials demonstrating that decreasing plasma homocyst(e)ine levels reduces the incidence of cardiovascular disease.

Therapies That Decrease Plasma Homocyst(e)ine Levels

Simple, safe, and inexpensive therapy can decrease homocyst(e)ine levels in most persons. Most patients respond to multivitamin treatment within 2 to 6 weeks of initiating therapy, irrespective of the cause of high homocyst(e)ine levels. Folic acid, alone or combined with vitamins B_6 and B_{12}, reduces plasma homocyst(e)ine levels even in persons who are not frankly vitamin deficient (91, 92). A recent meta-analysis of the effects of folic acid–based supplements on basal plasma homocyst(e)ine levels demonstrated that the proportional and absolute reductions in plasma homocyst(e)ine level produced by folic acid supplements were greater at higher pretreatment plasma homocyst(e)ine levels and at lower pretreatment blood folate levels (92).

Table 4—*Continued*

Age	Mean Follow-up	Major End Points	Total Homocysteine Level		Relative Risk or Odds Ratio (95% CI)
			Case-Patients	Controls	
y			*μmol/L*		
40–84	5	Fatal and nonfatal MI, CHD death	11.1	10.5	3.4 (1.3–8.8)†
35–64	8.7	Fatal CHD	≥15.2	<10.3	2.9 (2.04–4.1)‡
12–61	3.5	Fatal and nonfatal CHD	12.7	11.3	1.41 (1.16–1.71)§
40–59	12.8	Fatal and nonfatal stroke	13.7	11.9	2.8 (1.3–5.9)§
Median, 62	4.6	CHD death	≥20.0	<9.0	4.5 (1.22–16.6)‖
64–84	10	MI and stroke	–	–	1.81 (1.07–3.08) for MI¶ 4.61 (1.18–11.89) for stroke¶
Mean ± SD, 34.9 ± 11.7	4.8	Stroke and arterial thrombosis	–	–	2.44 (1.22–1.43) for stroke 3.49 (0.97–12.54) for arterial thrombosis
≥55	2.7	Stroke and MI	Stroke: 18.4; MI: 17.3	15.2	2.53 (1.19–5.35) for stroke** 2.43 (1.11–5.35) for MI**
40–84	7.5	Fatal and nonfatal MI, CHD death	–	–	1.7 (0.9–3.3)††
40–84	5	Ischemic stroke	11.4	10.6	1.2 (0.7–2.0)**
40–84	9	New angina, CABG	10.9	10.4	1.0 (0.4–2.4)‡‡
40–64	9	Fatal and nonfatal MI, stroke	Men: 9.9; women: 9.6	Men: 9.8; women: 9.3	Men: 1.05 (0.56–1.95); women: 1.22 (0.66–2.78)††
35–57	11–17	Nonfatal MI, CHD death	MI: 12.6; CHD death: 12.8	MI: 13.1; CHD death: 12.7	0.82 (0.55–1.54)‡
45–64	3.3	All CHD events	8.9	8.5	1.28 (0.5–3.2)**

Folic acid alone in a dosage of 0.5 to 5 mg/d was associated with an approximately 25% reduction (CI, 23% to 28%) in basal plasma homocyst(e)ine levels. The addition of vitamin B_{12} (mean dosage, 0.5 mg/d) was associated with an additional 7% reduction (CI, 3% to 10%) in basal plasma homocyst(e)ine level. The addition of vitamin B_6 (mean dosage, 16.5 mg/d) did not provide further benefit. However, this meta-analysis did not evaluate the effects of vitamin therapy on homocyst(e)ine levels after methionine loading, which may be more dependent on vitamin B_6.

The minimum daily dosage of folic acid that has maximal efficacy in decreasing plasma homocyst(e)ine levels is approximately 0.4 mg (24, 65, 92). Higher daily dosages are no more effective, except perhaps in patients with renal failure, and lower daily dosages of 0.1 mg seem to be inadequate. However, because the response to therapy to decrease homocyst(e)ine levels is not uniform and depends on such factors as genotype for enzymes involved in the metabolism of homocyst(e)ine and folate, the status of vitamins B_6 and B_{12}, and nutritional needs, multivitamin doses for the treatment of hyperhomocyst(e)inemia may need to be modified depending on the response of the individual patient.

A possible side effect of folic acid therapy is progressive neurologic damage (subacute combined degeneration of the spinal cord) in persons with subclinical vitamin B_{12} deficiency. In these patients, folic acid therapy may mask the development of the hematologic manifestations of this deficiency. However, this uncommon complication can be avoided by ruling out vitamin B_{12} deficiency before beginning folic acid therapy or by supplementing folic acid therapy with vitamin B_{12} therapy. A vitamin B_{12} dosage of 400 to 1000 μg/d is suggested because the recommended daily intake of vitamin B_{12} is approximately 2 μg and only 1% to 3% of oral vitamin B_{12} is absorbed by simple diffusion.

The major potential side effect of vitamin B_6 therapy is sensory neuropathy, a rare complication largely confined to patients who are treated for months to years with dosages exceeding 400 mg/d. The dosages of vitamin B_6 that are used to treat mildly and moderately elevated total plasma homocyst(e)ine levels are usually much lower than this (25 to 50 mg/d).

Since January 1998, the U.S. Food and Drug Administration has mandated that food products made with cereal or flour be fortified with 140 μg of folic acid per 100 g of flour for the prevention of neural tube defects; a similar policy was implemented in Canada in November 1998. Although a recent study (24) showed that a breakfast cereal providing 127 μg of folic acid per day reduced total plasma homocysteine levels by a mean of only 3.7%, it remains to be demonstrated what effect the widespread fortification of foods with folic acid would have on plasma homocyst(e)ine levels.

Planned and Ongoing Trials of Therapies To Decrease Homocysteine Levels for Cardiovascular Prevention

To date, no published randomized clinical trials have evaluated the effects of decreasing homocyst(e)ine levels on major cardiovascular events. The ability of randomized clinical trials to detect a treatment effect, if one exists, depends on the choice of the population, the specific treatment regimen, and the duration of therapy. Targeting persons at high risk for fatal and nonfatal cardiovascular events (that is, those with previous vascular disease); enrolling a sufficiently large sample; maximizing folic acid, vitamin B_6, and vitamin B_{12} doses; and allowing for a sufficiently long duration of treatment (which would permit the therapy to affect the atherosclerotic process and would allow a sufficiently large number of events to occur for a treatment effect to be shown) represent the best strategies in the design of randomized clinical trials evaluating the effects of decreasing homocyst(e)ine levels.

Several large randomized clinical trials of decreasing homocyst(e)ine levels are currently in progress (**Table 6**), including the Heart Outcomes Prevention Evaluation Study, which is funded by the Medical Research Council of Canada and will be conducted in many Canadian medical centers. In total, these trials are expected to include approximately 50 000 persons and will be able to detect

Table 5. Folate, Vitamins B_6 and B_{12}, and Cardiovascular Risk*

Study (Reference)	Design	Patient Selection	Setting	Participants	Age	Mean Follow-up	Main Results
					y		
de Bree et al. [Nutrition Canada Survey] (30)	Prospective cohort, nested case–control	5056 members of the general population	Canada	165 CHD deaths, men and women	35–79	15	RR, 1.69 (CI, 1.10–2.61) for lowest compared with highest quarters of folate level
Verhoef et al. [Physicians' Health Study] (80)	Prospective cohort, nested case–control	14 916 male physicians	United States	333 men after MI	40–84	7.5	RR, 1.4 (CI, 0.9–2.3) for lowest compared with highest fifths of folate level; RR, 1.5 (CI, 1.0–2.2) for lowest compared with highest fifths of vitamin B_6 level
Morrison et al. [Atherosclerosis Risk in Communities Study] (85)	Prospective cohort, nested case–control	15 792 members of the general population	United States	232 men and women with fatal and nonfatal CHD	45–64	3.3	RR, 0.66 (CI, 0.3–1.5) for highest compared with lowest fifths of folate level; RR, 0.28 (CI, 0.1–0.7) for highest compared with lowest fifths of vitamin B_6 level; RR, 0.81 (CI, 0.4–1.8) for highest compared with lowest fifths of B_{12} level
Giles et al. [National Health and Nutrition Examination Survey] (86)	Prospective cohort, nested case–control	14 407 members of the general population	United States	98 men and women with ischemic stroke	35–74	13	RR, 1.37 (CI, 0.82–2.29) for serum folate level $\leq$9.2 μmol/L compared with >9.2 μmol/L
Rimm et al. [Nurses' Health Study] (87)	Prospective cohort, nested case–control	80 082 female nurses	United States	658 women with nonfatal MI and CHD death	30–55	14	RR, 0.69 (CI, 0.55–0.87) for highest compared with lowest fifths of folate level; RR, 0.67 (CI, 0.53–0.85) for highest compared with lowest fifths of vitamin B_6 level
Robinson et al. [European Concerted Action Project] (88)	Retrospective case–control	–	Nine European countries	750 men and women with vascular disease, 800 controls	Mean ± SD, 47.2 ± 0.3	–	OR, 1.50 (CI, 1.03–2.20) for folate level <10th percentile; OR, 1.84 (CI, 1.39–2.42) for vitamin B_6 level <10th percentile; OR, 1.19 (CI, 0.80–1.76) for B_{12} level <10th percentile
Verhoef et al. [Boston Area Health Study] (89)	Retrospective case–control	–	United States	130 men and women with MI	Mean ± SD, 57.7 ± 9.3	–	$P = 0.03$ for folate level; $P = 0.005$ for vitamin B_6 level; P = NS for vitamin B_{12} level (folate, vitamin B_6, and vitamin B_{12} levels were lower in case-patients than in controls)

*CHD = coronary heart disease; MI = myocardial infarction; NS = not significant; OR = odds ratio; RR = relative risk.

Table 6. Randomized Clinical Trials of Therapy To Decrease Total Homocysteine Level in Patients with Vascular Disease*

Study (Reference)	Setting	Starting Date	Disease Studied	Intervention	Sample Size
Bergen Vitamin Study	Norway	1997	Stroke	2 × 2 factorial design: folic acid, 5 mg/d for 2 weeks, then 0.8 mg/d, compared with placebo; vitamin B_6, 40 mg/d, compared with placebo	2000
Cambridge Heart Antioxidant Study [CHAOS-2]	United Kingdom	1998	Myocardial infarction, unstable angina	Folic acid, 5 mg/d, compared with placebo	4000
Lonn et al. [Heart Outcomes Prevention Evaluation HOPE-2 Study] (94)	Canada	1999	Arterial vascular disease	Folic acid, 2.5 mg/d; vitamin B_6, 50 mg/d; plus vitamin B_{12}, 1 mg/d	5000
Norwegian Study of Homocysteine Lowering with B-Vitamins in Myocardial Infarction [NORVIT]	Norway	1998	Myocardial infarction	2 × 2 factorial design: folic acid, 5 mg/d for 2 weeks, then 0.8 mg/d, compared with placebo; vitamin B_6, 40 mg/d, compared with placebo	3000
Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease [PACIFIC] Study	Australia	1998†	Arterial vascular disease	2 × 2 factorial design: folic acid, 0.2 or 2 mg/d, compared with placebo; angiotensin-converting enzyme inhibitor compared with placebo	10 000
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine [SEARCH]	Oxford, United Kingdom	1998	Myocardial infarction	2 × 2 factorial design: folic acid, 2 mg/d, and vitamin B_{12}, 1 mg/d, compared with placebo; simvastatin, 80 mg/d, compared with 20 mg/d	12 000
Vitamins in Stroke Prevention [VISP] Trial	United States	1998	Stroke	Folic acid, 2.5 mg/d, vitamin B_6, 25 mg/d, and vitamin B_{12}, 0.4 mg/d, compared with folic acid, 0.02 mg/d, plus vitamin B_6, 0.2 mg/d, plus vitamin B_{12}, 0.06 mg/d	3600
VITAmins TO Prevent Stroke [VITATOPS] Study (95)	Australia	1999	Stroke	Folic acid, 2 mg/d, plus vitamin B_6, 25 mg/d, plus vitamin B_{12}, 400 μg/d, compared with placebo	5000
Women's Antioxidant and Cardiovascular Disease Study [WACS]	United States	1998	Vascular disease or high risk for vascular disease	Folic acid, 2.5 mg/d, plus vitamin B_6, 50 mg/d, plus vitamin B_{12}, 1 mg/d, compared with placebo	8000

* Data obtained in part from Clarke and Collins (93).
† A pilot study was initiated in 1998; the main PACIFIC trial will begin after completion of the pilot study.

even a 10% relative risk reduction in the incidence of major vascular events, such as death or myocardial infarction.

Conclusions

A substantial body of epidemiologic evidence suggests an association between cardiovascular risk and moderately increased plasma homocyst(e)ine levels. Although this association seems to be strong, dose related, independent of other risk factors, and biologically plausible, not all of the study results are consistent; it remains to be demonstrated that reducing elevated plasma homocyst(e)ine levels will result in a reduction in the risk for cardiovascular disease.

Large randomized trials are in progress to determine whether multivitamin therapy to decrease total homocysteine levels will reduce the risk for cardiovascular disease. If a combination of vitamins is found to be effective, this safe, inexpensive, easily administered therapy will probably be widely used throughout the world and have a major effect on public health.

From McMaster University, Hamilton, Ontario, Canada; Clinical Research Institute of Montreal, Montreal, Quebec, Canada; and Royal Perth Hospital, Perth, Western Australia.

Grant Support: Dr. Eikelboom is the recipient of a Medical Research Fellowship from the Haematology Society of Australia and the University of Western Australia. Dr. Yusuf is the recipient of a Medical Research Council of Canada Career Scientist Award and holds a Heart and Stroke Foundation of Ontario research chair.

Requests for Reprints: Eva Lonn, MD, Preventive Cardiology and Therapeutics Program, McMaster University, HGH, McMaster Clinic, 237 Barton Street East, Hamilton, Ontario L8L 2X2, Canada.

Current Author Addresses: Drs. Eikelboom, Lonn, and Yusuf: Preventive Cardiology and Therapeutics Program, McMaster University, HGH, McMaster Clinic, 237 Barton Street East, Hamilton, Ontario L8L 2X2, Canada.
Dr. Genest: Clinical Research Institute of Montreal, 110 avenue des Pins ouest, Montreal, Quebec H2W 1R7, Canada.
Dr. Hankey: Department of Neurology, Royal Perth Hospital, Wellington Street, Perth 6001, Australia.

References

1. Heart disease and stroke in Canada. 2. Deaths from cardiovascular disease. Laboratory Centre for Disease Control, Statistics Canada, 1997. http://www.hsf.ca/stats/stats-facts.htm. Accessed 22 March 1999.
2. **Goldman L, Cook EF.** The decline in ischemic heart disease mortality rates. An analysis of the comparative effects of medical interventions and changes in lifestyle. Ann Intern Med. 1984;101:825-36.

3. **Gordon T, Garcia-Palmieri MR, Kagan A, Kannel WB, Schiffman J.** Differences in coronary heart disease in Framingham, Honolulu and Puerto Rico. J Chronic Dis. 1974;27:329-44.
4. **Verschuren WM, Jacobs DR, Bloemberg BP, Kromhout D, Menotti A, Aravanis C, et al.** Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five year follow-up of the seven countries study. JAMA. 1995;274:131-6.
5. **Kannel WB, Castelli WP, Gordon T.** Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. Ann Intern Med. 1979;90:85-91.
6. Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking. A preliminary report from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. JAMA. 1990;264:3018-24.
7. **McGill HC Jr, McMahan CA, Malcom GT, Oalmann MC, Strong JP.** Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. The PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. Arterioscler Thromb Vasc Biol. 1997;17:95-106.
8. **Stamler J, Wentworth D, Neaton JD.** Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA. 1986;256:2823-8.
9. **Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB.** Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837-47.
10. **Rothman KJ.** Modern Epidemiology. Boston: Little, Brown; 1986:31-66.
11. **Boushey CJ, Beresford SA, Omenn GS, Motulsky AG.** A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA. 1995;274:1049-57.
12. **Finkelstein JD.** The metabolism of homocysteine: pathways and regulation. Eur J Pediatr. 1998;157(Suppl 2):S40-4.
13. **Ueland PM.** Homocysteine species as components of plasma redox thiol status [Editorial]. Clin Chem. 1995;41:340-2.
14. **Kraus JP.** Biochemistry and molecular genetics of cystathionine beta-synthase deficiency. Eur J Pediatr. 1998;157(Suppl 2):S50-3.
15. **Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, et al.** The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet. 1985;37:1-31.
16. **McCully KS, Wilson RB.** Homocysteine theory of arteriosclerosis. Atherosclerosis. 1975;22:215-27.
17. **Frantzen F, Faaren AL, Alfheim I, Nordhei AK.** Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. Clin Chem. 1998;44:311-6.
18. **Bostom AG, Jacques PF, Nadeau MR, Williams RR, Ellison RC, Selhub J.** Post-methionine load hyperhomocysteinemia in persons with normal fasting total homocysteine: initial results from the NHLBI Family Heart Study. Atherosclerosis. 1995;116:147-51.
19. **Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, et al.** Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA. 1997;277:1775-81.
20. **Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ.** The effect of blood sample aging and food consumption on plasma total homocysteine levels. Clin Chim Acta. 1992;207:119-28.
21. **Guttormsen AB, Schneede J, Fiskerstrand T, Ueland PM, Refsum HM.** Plasma concentrations of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. J Nutr. 1994;124:1934-41.
22. **Egerton W, Silberberg J, Crooks R, Ray C, Xie L, Dudman N.** Serial measures of plasma homocyst(e)ine after acute myocardial infarction. Am J Cardiol. 1996;77:759-61.
23. **McCully KS.** Homocysteine and vascular disease. Nat Med. 1996;2:386-9.
24. **Malinow MR, Duell PB, Hess DL, Anderson PH, Kruger WD, Phillipson BE, et al.** Reduction of plasma homocyst(e)ine levels by breakfast cereal fortified with folic acid in patients with coronary heart disease. N Engl J Med. 1998;338:1009-15.
25. **Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forde OH, Nordrehaug JE.** Serum total homocysteine and coronary heart disease. Int J Epidemiol. 1995;24:704-9.
26. **Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH.** Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA. 1993;270:2693-8.
27. **Ubbink JB, Vermaak WJ, van der Merwe A, Becker PJ.** Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. Am J Clin Nutr. 1993;57:47-53.
28. **Wilson JW, Enns CW, Goldman JD, Tippett KS, Mickle SJ, Cleveland LE, et al.** Data tables: combined results from USDA's 1994 and 1995 Continuing Survey of Food Intakes by Health Knowledge Survey. ARS Food Research Surveys Group. http://www.barc.usda.gov/bhnrc/foodsurvey/home.htm. Accessed 22 March 1999.
29. **Subar AF, Block G, James LD.** Folate intake and food sources in the US population. Am J Clin Nutr. 1989;50:508-16.
30. **de Bree A, van Dusseldorp M, Brouwer IA, van het Hof KH, Steegers-Theunissen RP.** Folate intake in Europe: recommended, actual and desired intake. Eur J Clin Nutr. 1997;51:643-60.
31. **Harker LA, Slichter SJ, Scott CR, Ross R.** Homocystinemia. Vascular injury and arterial thrombosis. N Engl J Med. 1974;291:537-43.
32. **Harker LA, Ross R, Slichter SJ, Scott CR.** Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. J Clin Invest. 1976;58:731-41.
33. **Wall RT, Harlan JM, Harker LA, Striker GE.** Homocysteine-induced endothelial cell injury in vitro: a model for the study of vascular injury. Thromb Res. 1980;18:113-21.
34. **Starkebaum G, Harlan JM.** Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest. 1986;77:1370-6.
35. **Blundell G, Jones BG, Rose FA, Tudball N.** Homocysteine mediated endothelial cell toxicity and its amelioration. Atherosclerosis. 1996;122:163-72.
36. **Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, et al.** Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest. 1993;91:308-18.
37. **Lentz SR, Sobey CG, Piegors DJ, Bhopatkar MY, Faraci FM, Malinow MR, et al.** Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. J Clin Invest. 1996;98:24-9.
38. **Celermajer DS, Sorensen K, Ryalls M, Robinson J, Thomas O, Leonard JV, et al.** Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. J Am Coll Cardiol. 1993;22:854-8.
39. **Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA.** Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. Circulation. 1997;95:1119-21.
40. **Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, et al.** Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci U S A. 1994;91:6369-73.
41. **Tsai JC, Wang H, Perrella MA, Yoshizumi M, Sibinga NE, Tan LC, et al.** Induction of cyclin A gene expression by homocysteine in vascular smooth muscle cells. J Clin Invest. 1996;97:146-53.
42. **Frauscher G, Karnaukhova E, Muehl A, Hoeger H, Lubec B.** Oral administration of homocysteine leads to increased plasma triglycerides and homocysteic acid—additional mechanisms in homocysteine induced endothelial damage? Life Sci. 1995;57:813-7.
43. **Halvorsen B, Brude I, Drevon CA, Nysom J, Ose L, Christiansen EN, et al.** Effect of homocysteine on copper ion-catalyzed, azo compound-initiated, and mononuclear cell-mediated oxidative modification of low density lipoprotein. J Lipid Res. 1996;37:1591-600.
44. **Blom HJ, Engelen DP, Boers GH, Stadhouders AM, Sengers RC, de Abreu R, et al.** Lipid peroxidation in homocysteinaemia. J Inherit Metab Dis. 1992;15:419-22.
45. **Rodgers GM, Kane WH.** Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. J Clin Invest. 1986;77:1909-16.
46. **Rodgers GM, Conn MT.** Homocysteine, an atherogenic stimulus, reduces protein C activation by arterial and venous endothelial cells. Blood. 1990;75:895-901.
47. **Lentz SR, Sadler JE.** Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. J Clin Invest. 1991;88:1906-14.
48. **Hayashi T, Honda G, Suzuki K.** An atherogenic stimulus homocysteine inhibits cofactor activity of thrombomodulin and enhances thrombomodulin expression in human umbilical vein endothelial cells. Blood. 1992;79:2930-6.
49. **Nishinaga M, Ozawa T, Shimada K.** Homocysteine, a thrombogenic agent, suppresses anticoagulant heparin sulfate expression in cultured porcine aortic endothelial cells. J Clin Invest. 1993;92:1381-6.
50. **Hajjar KA.** Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. J Clin Invest. 1993;91:2873-9.
51. **Welch GN, Loscalzo J.** Homocysteine and atherothrombosis. N Engl J Med. 1998;338:1042-50.
52. **Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al.** A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase [Letter]. Nat Genet. 1995;10:111-3.
53. **Arruda VR, von Zuben PM, Chiaparini LC, Annichino-Bizzacchi JM, Costa FF.** The mutation Ala677→Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. Thromb Haemost. 1997;77:818-21.
54. **Guttormsen AB, Ueland PM, Nesthus I, Nygard O, Schneede J, Vollset SE, et al.** Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia (> or = 40 micromol/liter). The Hordaland Homocysteine Study. J Clin Invest. 1996;98:2174-83.
55. **Brattstrom L, Wilcken DE, Ohrvik J, Brudin L.** Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation. 1998;98:2520-6.
56. **Malinow MR, Nieto FJ, Szklo M, Chambless LE, Bond G.** Carotid artery intimal-medial wall thickening and plasma homocyst(e)ine in asymptomatic adults. The Atherosclerosis Risk in Communities Study. Circulation. 1993;87:1107-13.
57. **Aronow WS, Ahn C, Schoenfeld MR.** Association between plasma homocysteine and extracranial carotid arterial disease in older persons. Am J Cardiol. 1997;79:1432-33.
58. **Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, et al.** Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arterioscler Thromb Vasc Biol. 1996;16:984-91.
59. **Clarke R, Fitzgerald D, O'Brien C, O'Farrell C, Roche G, Parker RA, et al.** Hyperhomocysteinaemia: a risk factor for extracranial carotid artery atherosclerosis. Ir J Med Sci. 1992;161:61-5.
60. **Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, et al.** Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med. 1995;332:286-91.

61. **Verhoef P, Kok FJ, Kruyssen DA, Schouten EG, Grobbee DE, Ueland PM, et al.** Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 1997;17:989-95.
62. **Montalescot G, Ankri A, Chadefaux-Vekemans B, Blacher J, Phillippe F, Drobinski G, et al.** Plasma homocysteine and the extent of atherosclerosis in patients with coronary artery disease. Int J Cardiol. 1997;60:295-300.
63. **van den Berg M, Stehouwer CD, Bierdrager E, Rauwerda JA.** Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. Arterioscler Thromb Vasc Biol. 1996;16:165-71.
64. **Konecky N, Malinow MR, Tunick PA, Freedberg RS, Rosenzweig BP, Katz ES, et al.** Correlation between plasma homocyst(e)ine and aortic atherosclerosis. Am Heart J. 1997;133:534-40.
65. **Markus HS, Ali N, Swaminathan R, Sankaralingam A, Molloy J, Powell J.** A common polymorphism in the methylenetetrahydrofolate reductase gene, homocysteine, and ischemic cerebrovascular disease. Stroke. 1997;28:1739-43.
66. **Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johansson BB.** Plasma homocysteine in the acute and convalescent phases after stroke. Stroke. 1995;26:795-800.
67. **Hopkins PN, Wu LL, Wu J, Hunt SC, James BC, Vincent GM, et al.** Higher plasma homocyst(e)ine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease. Arterioscler Thromb Vasc Biol. 1995;15:1314-20.
68. **Dalery K, Lussier-Cacan S, Selhub J, Davignon J, Latour Y, Genest J Jr.** Homocysteine and coronary artery disease in French Canadian subjects: relation with vitamins B_{12}, B_6, pyridoxal phosphate, and folate. Am J Cardiol. 1995;75:1107-11.
69. **Robinson K, Mayer EL, Miller DP, Green R, van Lente F, Gupta A, et al.** Hyperhomocysteinemia and low pyridoxal phosphate. Common and independent reversible risk factors for coronary artery disease. Circulation. 1995; 92:2825-30.
70. **Malinow MR, Ducimetiere P, Luc G, Evans AE, Arveiler D, Cambien F, et al.** Plasma homocyst(e)ine levels and graded risk for myocardial infarction: findings in two populations at contrasting risk for coronary heart disease. Atherosclerosis. 1996;126:27-34.
71. **Alfthan G, Aro A, Gey KF.** Plasma homocysteine and cardiovascular disease mortality [Letter]. Lancet. 1997;349:397.
72. **Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, et al.** A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA. 1992;268:877-81.
73. **Wald NJ, Watt HC, Law MR, Weir DG, McPartlin J, Scott JM.** Homocysteine and ischemic heart disease: results of a prospective study with implications regarding prevention. Arch Intern Med. 1998;158:862-7.
74. **Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG.** Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet. 1995;346:1395-8.
75. **Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE.** Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 1997;337:230-6.
76. **Stehouwer CD, Weijenberg MP, van den Berg M, Jakobs C, Feskens EJ, Kromhout D.** Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly men: a 10-year follow-up. Arterioscler Thromb Vasc Biol. 1998;18:1895-901.
77. **Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J, Rosenberg IH.** Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet. 1996;348:1120-4.
78. **Bots ML, Launer LJ, Lindemans J, Hoes AW, Hofman A, Witteman JC, et al.** Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam study. Arch Intern Med. 1999;159:38-44.
79. **Chasan-Taber L, Selhub J, Rosenberg IH, Malinow MR, Terry P, Tishler PV, et al.** A prospective study of folate and vitamin B_6 and risk of myocardial infarction in US physicians. J Am Coll Nutr. 1996;15:136-43.
80. **Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ.** A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. Stroke. 1994;25:1924-30.
81. **Verhoef P, Hennekens CH, Allen RH, Stabler SP, Willett WC, Stampfer MJ.** Plasma total homocysteine and risk of angina pectoris with subsequent coronary artery bypass surgery. Am J Cardiol. 1997;79:799-801.
82. **Alfthan G, Pekkanen J, Jauhiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J, et al.** Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis. 1994;106:9-19.
83. **Evans RW, Shaten BJ, Hempel JD, Cutler JA, Kuller LH.** Homocyst(e)ine and risk of cardiovascular disease in the Multiple Risk Factor Intervention Trial. Arterioscler Thromb Vasc Biol. 1997;17:1947-53.
84. **Folsom AR, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, et al.** Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 1998;98: 204-10.
85. **Morrison HI, Schaubel D, Desmeules M, Wigle DT.** Serum folate and risk of fatal coronary heart disease. JAMA. 1996;275:1893-6.
86. **Giles WH, Kittner SJ, Anda RF, Croft JB, Casper ML.** Serum folate and risk for ischemic stroke. First National Health and Nutrition Examination Survey epidemiologic follow-up study. Stroke. 1995;26:1166-70.
87. **Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, Manson JE, et al.** Folate and vitamin B_6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA. 1998;279:359-64.
88. **Robinson K, Arheart K, Refsum H, Brattstrom L, Boers G, Ueland P, et al.** Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. Circulation. 1998;97:437-43.
89. **Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, et al.** Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B_6, B_{12}, and folate. Am J Epidemiol. 1996;143:845-59.
90. **Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC.** Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease in men. JAMA. 1996;275:447-51.
91. **den Heijer M, Brouwer IA, Bos GM, Blom HJ, van der Put NM, Spaans AP, et al.** Vitamin supplementation reduces blood homocysteine levels: a controlled trial in patients with venous thrombosis and healthy volunteers. Arterioscler Thromb Vasc Biol. 1998;18:356-61.
92. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. BMJ. 1998;316:894-8.
93. **Clarke R, Collins R.** Can dietary supplements with folic acid or vitamin B6 reduce cardiovascular risk? Design of clinical trials to test the homocysteine hypothesis of vascular disease. J Cardiovasc Risk. 1998;5:249-55.
94. **Lonn E, Genest J, Yusuf S, Pogue J, Liu P, McQueen M, Title L, et al.** Heart Outcomes Prevention Evaluation (HOPE-2) Study, McMaster University, Canada. Medical Research Council of Canada Grant number MT-15428.
95. VITATOPS Study Group. Vitamins to Prevent Stroke (VITATOPS) Study. Stroke Unit, Royal Perth Hospital, University of Western Australia, Australia.