Homocysteine Level and Coronary Heart Disease Incidence: A Systematic Review and Meta-analysis

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OBJECTIVE: **To determine whether an elevated homocysteine level is an independent risk factor for the development of coronary heart disease (CHD) to aid the US Preventive Services Task Force in its evaluation of novel risk factors for incident CHD.**

METHODS: **Studies of homocysteine and CHD were identified by searching MEDLINE (1966 through March 2006). We obtained additional articles by reviewing reference lists from prior reviews, original studies, editorials, and Web sites and by consulting experts. We included prospective cohort studies that measured homocysteine and Framingham risk factors and the incidence of CHD in the general adult population without known CHD. Each study was quality rated using criteria developed by the US Preventive Services Task Force. We conducted a meta-analysis using a random-effects model to determine summary estimates of the risk of major CHD associated with each 5-µmol/L increase in homocysteine level. The systematic review and meta-analysis were conducted between January 25, 2005, and September 17, 2007.**

RESULTS: **We identified 26 articles of good or fair quality. Most studies found elevations of 20% to 50% in CHD risk for each increase of 5 µmol/L in homocysteine level. Meta-analysis yielded a combined risk ratio for coronary events of 1.18 (95% confidence interval, 1.10-1.26) for each increase of 5 µmol/L in homocysteine level. The association between homocysteine and CHD was similar when analyzed by sex, length of follow-up, outcome, study quality, and study design.**

CONCLUSION: **Each increase of 5 µmol/L in homocysteine level increases the risk of CHD events by approximately 20%, independently of traditional CHD risk factors.**

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CHD = coronary heart disease; CI = confidence interval; CVD = cardiovascular disease; MI = myocardial infarction; RR = risk ratio; USPSTF = US Preventive Services Task Force

Coronary heart disease (CHD) and cardiovascular disease (CVD) are the leading causes of death each year in the United States and Europe, accounting for nearly 40% of all deaths.[1] Several important risk factors are recognized and have been formally encompassed in the Framingham risk prediction tool.[2,3] Although traditional risk factors such as hyperlipidemia, smoking, hypertension, and diabetes mellitus are thought to explain most CHD,[2,4,5] 15% to 20% of those with CHD have no identified risk factors and miss the opportunity for primary prevention.[5] For this reason, epidemiologists and biologists have tried to identify other risk factors, particularly modifiable risk factors, that predict a portion of CHD events and might improve primary prevention efforts. Elevated plasma homocysteine level is a suggested and biologically plausible candidate.

Interest in homocysteine as a causal factor was spurred by the observation that more than 50% of children with the genetic disorder homocystinuria die of premature vascular disease,[6,7] and strategies that reduce homocysteine levels in these children have been shown to decrease vascular event rates.[7,8] In mammals, severe elevations of homocysteine levels due to inborn errors of metabolism are associated with several physiologic abnormalities that might explain increased vascular risk.[9,10] In humans, moderate elevations of homocysteine levels can occur because of less severe genetic mutations associated with enzyme abnormalities in the metabolic pathway involving folate and homocysteine. However, inadequate intake of folate and vitamins B_6 and B_{12}, which play an important role in homocysteine metabolism, causes most elevations of homocysteine levels in the United States. Data from US prevalence studies have shown that elevations occur in a substantial proportion of the population, that many US adults are not consuming enough folate to keep their homocysteine levels within the normal range,[11-14] and that folic acid intake varies significantly by age, race, and ethnicity.[15]

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We undertook this review and meta-analysis to aid the US Preventive Services Task Force (USPSTF) in its evaluation of novel risk factors for the development of CHD. Although observational studies have fairly consistently shown that mild to moderate elevations of homocysteine levels are associated with a slight increase in CHD risk,[16] the recent publication of 2 randomized controlled trials showing no benefit for folate and vitamin B therapy among

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patients with *established* CHD[17,18] has diminished enthusiasm for screening in the general population to identify those who have elevated homocysteine levels.[19] These results in patients with known CVD have little bearing on the question addressed in our review: whether testing for homocysteine might refine estimates of coronary risk among those who are classified as intermediate risk by the Framingham risk scoring system. Such testing might identify persons who are at higher risk than predicted by traditional CHD risk factors and who might benefit from more aggressive management of traditional risk factors to prevent CHD. We specifically investigated whether adding homocysteine levels to current risk prediction models might identify patients who would benefit from more aggressive management of traditional risk factors, such as hyperlipidemia, the treatment of which has been shown to reduce CHD risk.

Previous meta-analyses[16,20,21] of observational studies have provided less than definitive answers to the question of whether elevations of homocysteine levels might provide incremental predictive value for CHD events if added to current methods of risk assessment based on traditional risk factors. These meta-analyses have been limited by the inclusion of cross-sectional studies, studies conducted among patients with known CHD, and case-control studies that are typically conducted among patients with prevalent CHD. Furthermore, because elevated homocysteine levels are also associated with known CHD risk factors, such as hypertension,[16] male sex,[22] renal dysfunction,[23] and smoking,[16] many prior studies and meta-analyses are limited by the inclusion of studies with insufficient adjustment for factors that might confound the association between CHD and elevated homocysteine levels. Our meta-analysis aimed to evaluate only prospective studies among the general population without known CHD and to carefully assess evaluation of traditional risk factors by contributing studies.

METHODS

We reviewed the MEDLINE and Cochrane Controlled Trials Register Database (1966 through March 2006) using the following keywords: *cohort studies*, *cardiovascular diseases*, *homocysteine*, *hyperhomocysteinemia*, and *cystathionine beta-synthase*. To ensure complete ascertainment, we reviewed the bibliographies of reviews, editorials, book chapters, and letters that discussed the association between homocysteine and CHD outcomes. We sought primary prevention trials and studies that evaluated the risk associated with elevated homocysteine levels in the general population. Criteria for inclusion in the systematic review and meta-analysis were the following: randomized controlled trials of primary prevention and/or prospective studies with cohort or nested case-control design, CHD or CVD as an outcome of interest, and availability of English-language abstract for review. We included studies that explicitly excluded persons with known CHD or CVD as well as those conducted in the general population without clear exclusion of persons with CHD. We excluded studies in which all participants had prevalent CHD or CVD.

The primary investigator (L.L.H.) abstracted study data and compiled evidence tables. Studies varied in the methods used to measure homocysteine levels, and only those that did so without methionine loading were included in this review. The studies also varied in the outcomes they evaluated. For the purposes of this review, CHD events include CHD death, myocardial infarction (MI), and revascularization procedures and CVD events include total CHD events and stroke. Myocardial infarction represents a subset of CHD events, and studies generally included electrocardiographic and enzyme abnormalities in defining MI. In most cases, studies defined CVD mortality by *International Classification of Disease* codes or World Health Organization criteria or included any death resulting from CHD or CVD. When there were multiple publications from a single cohort, we included the analysis with the highest applicability to the key questions and the highest internal validity on the basis of our quality ratings, using a best-evidence approach.[24]

To assess the validity of each of these studies, we reviewed all related articles describing the studies but did not query the study authors. Two investigators (L.L.H., K.R.) independently rated the quality of each study on the basis of criteria created by the third USPSTF[25] (Table 1); discrepancies were adjudicated. After reviewing and rating the studies, we limited our formal review and meta-analyses to only studies rated as fair or good quality. In our quality ratings, we gave great importance to control for CHD risk factors because our goal was to identify whether homocysteine level is an independent risk factor for CHD.

The focus of our systematic review and meta-analysis, conducted between January 25, 2005, and September 17, 2007, was CHD events. However, in some circumstances, studies reported only a combined estimate for all CVD. Because most atherosclerotic CVD is accounted for by CHD,[26] when no other data were available, we included CVD estimates in our review and meta-analysis. Reported CHD outcomes were categorized into 2 groups: CHD events and CVD events (as defined herein). We then grouped studies by whether persons with baseline CHD were explicitly excluded from the cohort.

Meta-analysis was performed to estimate the association between homocysteine level and CHD after controlling for both traditional and other risk factors. The traditional risk factors considered were age, sex, smoking, diabetes, hyper-

TABLE 1. **US Preventive Services Task Force Quality Rating Criteria**

Randomized controlled trials (RCTs) and cohort studies

Criteria
- Initial assembly of comparable groups: RCTs—adequate randomization, including concealment and whether potential confounders were distributed equally among groups; cohort studies—consideration of potential confounders with either restriction or measurement for adjustment in the analysis; consideration of inception cohorts
- Maintenance of comparable groups (includes attrition, crossovers, adherence, contamination)
- Important differential loss to follow-up or overall high loss to follow-up
- Measurements: equal, reliable, and valid (includes masking of outcome assessment)
- Clear definition of interventions
- Important outcomes considered

Analysis: adjustment for potential confounders for cohort studies or intention-to-treat analysis for RCTs (ie, analysis in which all participants in a trial are analyzed according to the intervention to which they were allocated, regardless of whether or not they completed the intervention)

Definition of ratings on the basis of above criteria
- Good: Meets all criteria: comparable groups are assembled initially and maintained throughout the study (follow-up at least 80%); reliable and valid measurement instruments are used and applied equally to the groups; interventions are spelled out clearly; important outcomes are considered; and appropriate attention is given to confounders in analysis
- Fair: Any or all of the following problems occur, without the important limitations noted in the "poor" category: generally comparable groups are assembled initially but some question remains about whether some (although not major) differences occurred in follow-up; measurement instruments are acceptable (although not the best) and are generally applied equally; some but not all important outcomes are considered; and some but not all potential confounders are accounted for
- Poor: Any of the following major limitations exists: groups assembled initially are not close to being comparable or maintained throughout the study; unreliable or invalid measurement instruments are used or not applied at all equally among groups (including not masking outcome assessment); and key confounders are given little or no attention

Case-control studies

Criteria
- Accurate ascertainment of cases
- Nonbiased selection of cases/controls with exclusion criteria applied equally to both
- Response rate
- Diagnostic testing procedures applied equally to each group
- Measurement of exposure accurate and applied equally to each group
- Appropriate attention to potential confounding variables

Definition of ratings on the basis of the above criteria
- Good: Appropriate ascertainment of cases and nonbiased selection of case and control participants; exclusion criteria applied equally to cases and controls; response rate equal to or greater than 80%; diagnostic procedures and measurements accurate and applied equally to cases and controls; and appropriate attention to confounding variables
- Fair: Recent, relevant, without major apparent selection or diagnostic work-up bias but with response rate less than 80% or attention to some but not all important confounding variables
- Poor: Major selection or diagnostic work-up biases, response rates less than 50%, or inattention to confounding variables

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tension, and hyperlipidemia. Studies reported risk ratios (RRs) on the basis of different cutoff levels of homocysteine, including median, tertiles, quartiles, or quintiles, or as a continuous risk. To standardize the RRs, we assumed a log-linear association between CHD risk and homocysteine level[27-30] and estimated for each study the RR associated with a 5-μmol/L increase in homocysteine.

Heterogeneity was assessed by standard χ^2 tests. The RRs were combined using a random-effects model to account for variation among studies. When no variation is found among studies, the random-effects model yields the same results as a fixed-effects model. Subgroup analyses were performed on study-level variables, such as mean duration of the study, study design (cohort vs nested case-control), outcome (MI, CHD events, and CVD events), quality rating, and sex. We calculated summary estimates separately for studies that explicitly excluded CHD and those conducted in the general population without clear exclusion of persons with CHD. Publication bias was evaluated by funnel plot and the linear regression method used by Egger et al,[31] and none was detected. All analyses were performed with STATA statistical software, version 9.1 (StataCorp, College Station, TX).

RESULTS

A total of 603 abstracts were identified from our literature searches, and 163 articles were reviewed for inclusion. Of the 31 studies (articles) from 24 cohorts representing populations from North America and Europe that were included (Table 2), 23 were nested case-control studies,[27,28,30,32-50,58] and 8 were cohort studies.[29,51-57] Of the 23 nested case-control studies, 12 studies were rated good quality,[28,35-38,41,44-48,53] 6 fair quality,[32-34,39,42,43] and 5 poor quality[27,30,40,49,50] on the basis of the USPSTF criteria for judging study quality. Two of the 8 cohort studies were rated good quality[51,53] and the other 6 fair quality[29,49,52,55-57] (Figure 1). Most cohorts were population based and had good external validity. The

TABLE 2. **Studies of Homocysteine and CHD**[a]

Study/reference	Study design, population, age (y)	Sample size, cases/controls	Follow-up (y)	Outcomes evaluated	Variables	Quality
ARIC/Folsom et al,[38] 1998	Nested case-control, men and women, 45-64	232/537	3.1	Silent and symptomatic MI; CHD death; coronary revascularization	Age, sex, TC, HDL-C, BP, DM, smoking, race, center	Good
British Regional Heart Study/Whincup et al,[50b] 1999	Nested case-control, men, 40-59 (mean, approximately 52)	386/454	12.8	First major fatal/nonfatal CHD event	Age, TC, HDL-C, BP, DM, smoking, BMI, exercise, alcohol, FEV_1, creatinine, urate	Good
BUPA/Wald et al,[30b] 1998	Nested case-control, men, 53 (median)	229/1126	8.7	CHD death	Age, sex, BP, apoprotein B	Poor
Caerphilly/Ubbink et al,[54] 1998; Fallon et al,[37c] 2001	Nested case-control, men, 45-59	312/1248	10.0	CHD death; clinical or silent MI	Age, sex, TC, HDL-C, BP, DM, smoking, race, center	Good
Framingham/Bostom et al,[51] 1999	Cohort, men and women, 71 (mean)	1933	10.0	CVD death; total mortality	Sex, age, DM, smoking, systolic BP, TC, LDL-C	Good
France/Blacher et al,[27b] 2002	Nested case-control, men and women, 65 (mean)	110/154	14.0	Fatal CVD	Age, sex, BP, CRP, DM, prevalent CHD	Poor
Gothenburg/Zylberstein et al,[57] 2004	Cohort, women, 38-60	1368	24.0	Fatal and nonfatal MI	Age, smoking, BMI, weight-height ratio, TG, cholesterol, BP, coffee, folate, creatinine, vitamin B_{12}	Fair
Hordaland/Nurk et al,[52b] 2002; Vollset et al,[55c] 2001	Cohort, men and women, 40-42 and 67-67, respectively	17/361	5.3	CVD hospitalization; CVD mortality	Sex, age, smoking, DM, cholesterol, BMI, systolic BP, hypertension	Fair
Hoorn/ Hoogeveen et al,[39b] 2000	Nested case-control, men and women, 50-75	171/640	5.0	CVD death	Age, sex, TC, BP, DM, smoking, HbA_{1c}, albumin	Poor
Kuopio/Voutilainen et al,[49b,d] 2000; Voutilainen et al,[56] 2004	Cohort study, men, 46-64	2682	Approximately 8.0	Acute coronary events	Age, examination year, systolic BP, smoking, BMI, LDL-C, HDL-C	Poor-fair
Malmo/Lind et al,[43] 2003	Nested case-control, men, 48 (mean)	241/241	17.0	Fatal or nonfatal MI	Age, sex, TC, BP, DM, smoking	Fair
Mobile Clinic-Finland/ Knekt et al,[41] 2001	Nested case-control, women, 45-64	75/149	13.0	Nonfatal MI and CHD deaths	Age, sex, TC, BP, DM, smoking, BMI	Good
Mobile Clinic-Finland/ Knekt et al,[42] 2001	Nested case-control, men, 45-64	272/524	13.0	Nonfatal or fatal CHD	Age, sex, TC, BP, DM, smoking, alcohol use, BMI	Fair
MONICA/VIP/Hultdin et al,[40b] 2004	Nested case-control, men and women, 53 (mean)	50/56	8.4	MI by WHO/ MONICA criteria	Age, sex, creatinine, albumin	Poor
Monitoring Project on CVD Risk Factors/ de Bree et al,[35] 2003	Nested case-control, men and women, 20-59	170/749	10.3	CHD death	Age, sex, TC, HDL-C, BP, smoking, creatinine, study center	Good
MRFIT/Evans et al,[36] 1997	Nested case-control, men, 35-57	240/472	11.0-17.0	Nonfatal MI; CHD death	Age, smoking, clinic, race, HDL-C, LDL-C, TG, diastolic BP	Good
North Kareleia/Alfthan et al, [33] 1994	Nested case-control, men and women, 30-64	191/269 191/269	9.0 9.0	Fatal and nonfatal MI	Age, sex, TC systolic BP, smoking	Fair
Northern Manhattan Study/Sacco et al,[53] 2004	Cohort study, men and women, 69 (mean)	2939	5.0	MI; vascular death	Age, race, sex, education, hypertension, DM, cardiac disease, HDL-C, alcohol, smoking, renal insufficiency, vitamin B_{12}	Good
Nurses Health Study/Shai et al,[46] 2004	Nested case-control, US female nurses, 30-55	237/458	8.0	Fatal CHD; nonfatal MI	Age, smoking, hours fasting, year Hcy, BMI, parental MI at <60 y, hypertension, DM, heart rate, alcohol, activity, HDL-C, TC, CRP	Good

(Continued)

TABLE 2. **Continued**[a]

Study/reference	Study design, population, age (y)	Sample size, cases/controls	Follow-up (y)	Outcomes evaluated	Variables	Quality
PHS/Albert et al,[32c,e] 2002; Verhoef et al,[48c,e] 1997; Stampfer et al,[47] 1992; Chasan-Taber et al,[58c] 1996	Nested case-control, US male physicians, 40-84 (mean, 59)	149/149	9.0	Sudden cardiac death;new angina and subsequent CABG; acute MI or CHD death	TC, HDL-C, BP, DM, BMI, alcohol use, aspirin	Good
Rotterdam/Bots et al,[28] 1999	Nested case-control, men and women, ≥55	224/533	2.7	Definite or probable MI	Age, sex, TC, HDL-C, BP, DM, smoking, prevalent CHD or stroke	Good
Tromso Health Study/ Arnesen et al,[34] 1995	Nested case-control, men and women, 12-61	122/478	4.0	Fatal and nonfatal CHD	Survey date, age, sex, hours since last meal, TC, HDL-C, BP, DM, TG, smoking, angina	Fair
Women's Health Study/ Ridker et al,[45c] 1999; Ridker et al,[44] 2000	Nested case-control, postmenopausal women, 59.3 (mean)	122/244	3.0	MI; stroke; CHD death; coronary revascularization	Age, sex, TC, HDL-C, BP, DM, smoking, BMI, aspirin, vitamin E, family history, CVD, CRP, Lp(a)	Good
Zutphen Elderly Study/ Stehouwer et al,[29] 1998	Cohort study, men, 64-84 (mean, 71)	878	≥9.0	First MI; CHD death	Age, BMI, BP, HDL-C, TC, DM, smoking	Fair

[a] ARIC = Atherosclerosis Risk in Communities; BMI = body mass index; BP = blood pressure; BUPA = British United Provident Association; CABG = coronary artery bypass graft; CHD = coronary heart disease; CRP = C-reactive protein; CVD = cardiovascular disease; DM = diabetes mellitus; FEV_1 = forced expiratory flow in 1 second; HbA_{1c} = hemoglobin A_{1c}; Hcy = homocysteine measured; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein a; MI = myocardial infarction; MONICA = Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; MRFIT = Multiple Risk Factor Intervention Trial; PHS = Public Health Service; TC = total cholesterol; TG = triglycerides; VIP =Västerbotten Intervention Project; WHO = World Health Organization.

[b] Not included in meta-analysis because of poor quality.

[c] Not included in meta-analysis because of duplicate study.

[d] Only data from ages 65 to 67 years.

[e] Not included in meta-analysis because outcome not clearly CHD or CVD.

cohort sizes were approximately 200 to 22,000 persons, with follow-up ranging from 1 to 25 years. Some studies were conducted only among men or women or were analyzed by sex; some combined data into sex-adjusted summary relative risks.

Our review focused on the 26 articles of good or fair quality. These articles include data collected among 20 cohorts; 5 (of the 26) articles were not used in the meta-analysis because the outcome studied could not be clearly attributed to CHD (eg, chronic heart failure)[32] or data were included in another publication.[37,45,48,58] Of the 21 remaining studies, 11 showed or suggested a positive association between elevated homocysteine level and incident CHD, 5 suggested no effect or a protective one, and 5 could be described as borderline (Figure 2). Although the test for heterogeneity was not statistically significant (Q=29.23; df =21; P=.11), we explored several possible explanations for the variation in the RRs.

When all fair or good quality studies were combined in a random-effects meta-analysis, the estimated RR for CHD events (as defined herein) associated with each 5-μmol/L increase in homocysteine was 1.18 (95% confidence interval [CI], 1.10-1.26) (Figure 2). Although the RRs appear to be inconsistent, the upper end of the CI overlaps the point estimate of the pooled result in all the studies that did not show an association (Figure 2). Elevated homocysteine levels were a stronger risk factor in studies in which persons with CHD were explicitly excluded (RR, 1.21; 95% CI, 1.10-1.32; Figure 2) than in studies in which exclusion was not described (RR, 1.09; 95% CI, 1.01-1.18; Figure 2). Inclusion of 5 studies rated poor quality[27,30,40,49,50] did not change the result (RR, 1.19; 95% CI, 1.12-1.27).

Pooled results were similar among the good and fair quality studies and did not vary by year of publication, study design (nested case-control vs cohort study), outcome measure, or number of Framingham risk factors for which adjustments were made. The association of homocysteine level with incident CHD was stronger in studies with follow-up of less than 5 years (RR, 1.39; 95% CI, 1.20-1.62) than in studies of 5 to 10 years' duration (RR, 1.13; 95% CI, 1.02-1.26) or of more than 10 years' duration (RR, 1.13; 95% CI, 1.00-1.28). Among studies that excluded patients who had CHD, 4 of 4 studies with a duration less than 5 years, 3 of 6 with a duration of 5 to 10 years, and 1 of 5 with a duration longer than 10 years had a RR greater than the pooled estimate of 1.21.

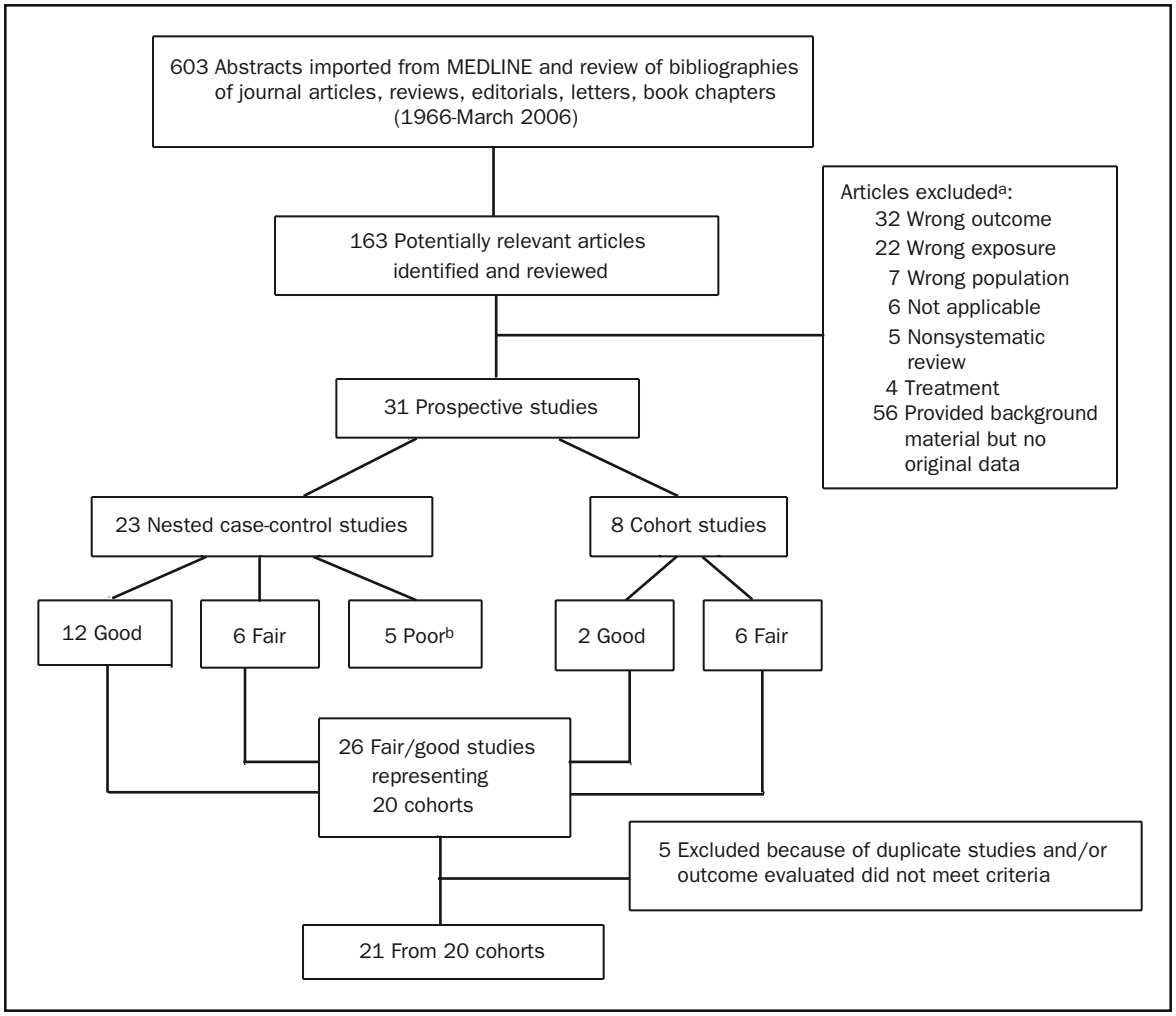

FIGURE 1. Search and selection of literature on homocysteine.
[a] Met multiple exclusion criteria.
[b] Excluded from meta-analysis.

All 7 studies involving only women or subgroup analyses conducted among women showed either a statistically significant association[41,44,57] or a trend[33-35,46] between higher homocysteine levels and CHD events. When combined in meta-analysis, the estimated RR among studies that included women was 1.21 (95% CI, 1.06-1.37) for each 5-μmol/L increase in homocysteine level. For studies that included only men, it was 1.13 (95% CI, 1.00-1.27).

DISCUSSION

Our review shows an association between elevated homocysteine levels and CHD that is independent of Framingham risk factors. In the overall analysis, the risk of any CHD event increased approximately 20% for each increase of 5 μmol/L of homocysteine. Because some studies reported only CVD events and because statistically these events are dominated by CHD events, we think that the overall estimate of 1.18 for all good and fair studies combined gives an accurate measure of the risk of CHD.

To our knowledge, this is the first meta-analysis to evaluate homocysteine levels as a predictor of new CHD events in persons without known CHD. Prior meta-analyses, which included persons with known CVD, have found similar increased risks of CHD associated with higher homocysteine levels.[16,20,21]

We do not think the association between higher levels of homocysteine and CHD was due to unrecognized confounding by factors associated with homocysteine levels and CHD. Elevations of homocysteine levels are associated with other important risk factors for CHD, such as hypertension,[59,60] smoking,[60] renal dysfunction, physical activity,[60] and male sex.[60] Although most of the studies we reviewed adjusted for traditional risk factors, most did not

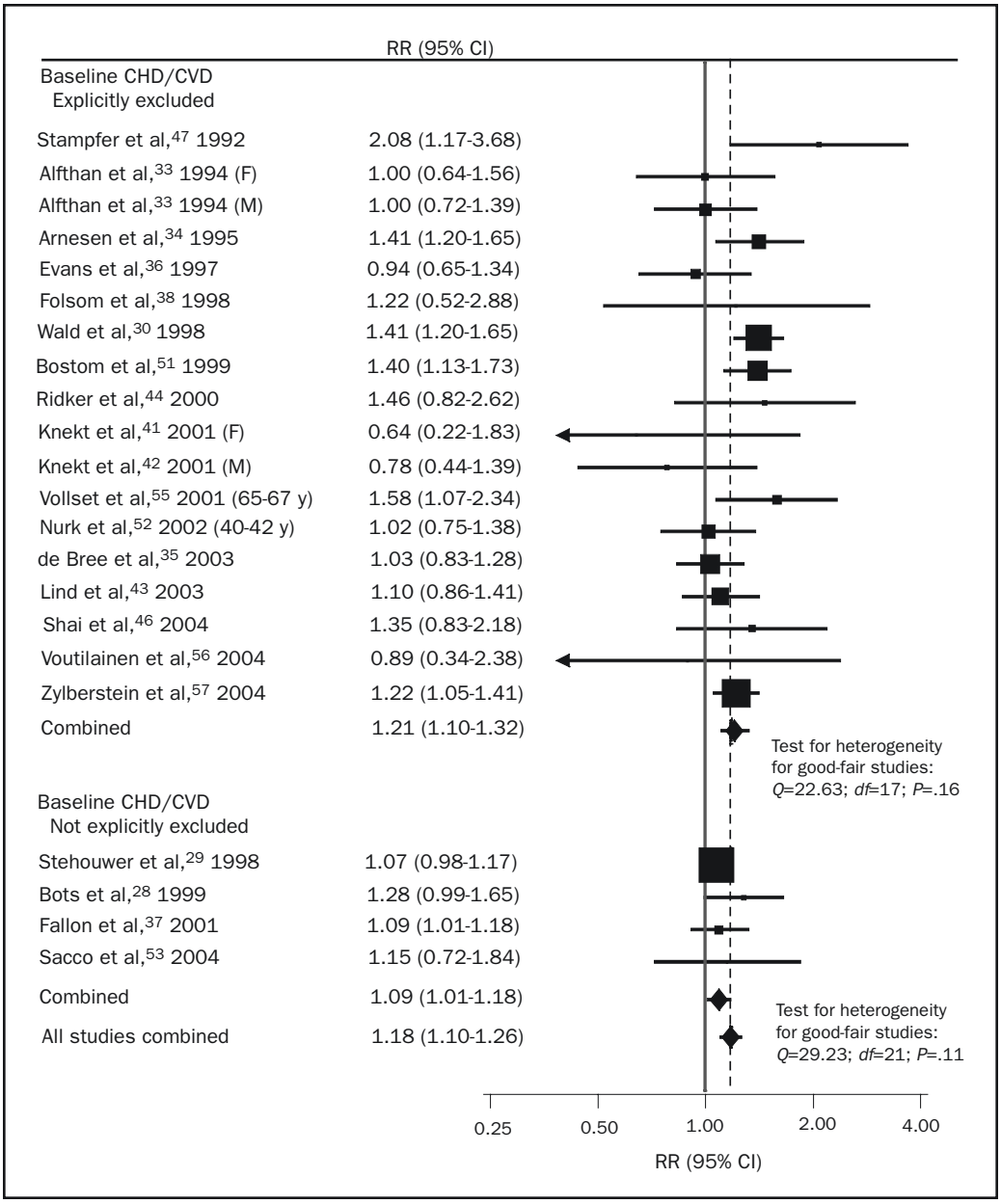

FIGURE 2. Study characteristics and risk ratio (RR) of coronary heart disease (CHD) associated with every 5-µmol/L increase of homocysteine level. CI = confidence interval; CVD = cardiovascular disease; F = female; M = male.

adjust for renal dysfunction, which has been shown to be a confounder in several studies.[61,62] However, the good quality Framingham study,[51] as well as the Rotterdam[28] and Gothenburg[57] studies, found no change in relative risk estimates when measures of renal function were added to the multivariable models. We also found little evidence of differences in relative risk estimates between studies with many adjustments for traditional risk factors vs those with fewer such adjustments (Figure 3).

This review has several limitations. We relied only on published data, which may be biased toward publishing positive findings. However, a funnel plot used to evaluate this possibility did not suggest publication bias. A further limitation of using only published data was that the outcomes evaluated were not standardized among studies, and we relied on each study's definition of the outcome. For example, as indicated in Table 2, some studies evaluated only MI (defined variably) as an outcome, whereas

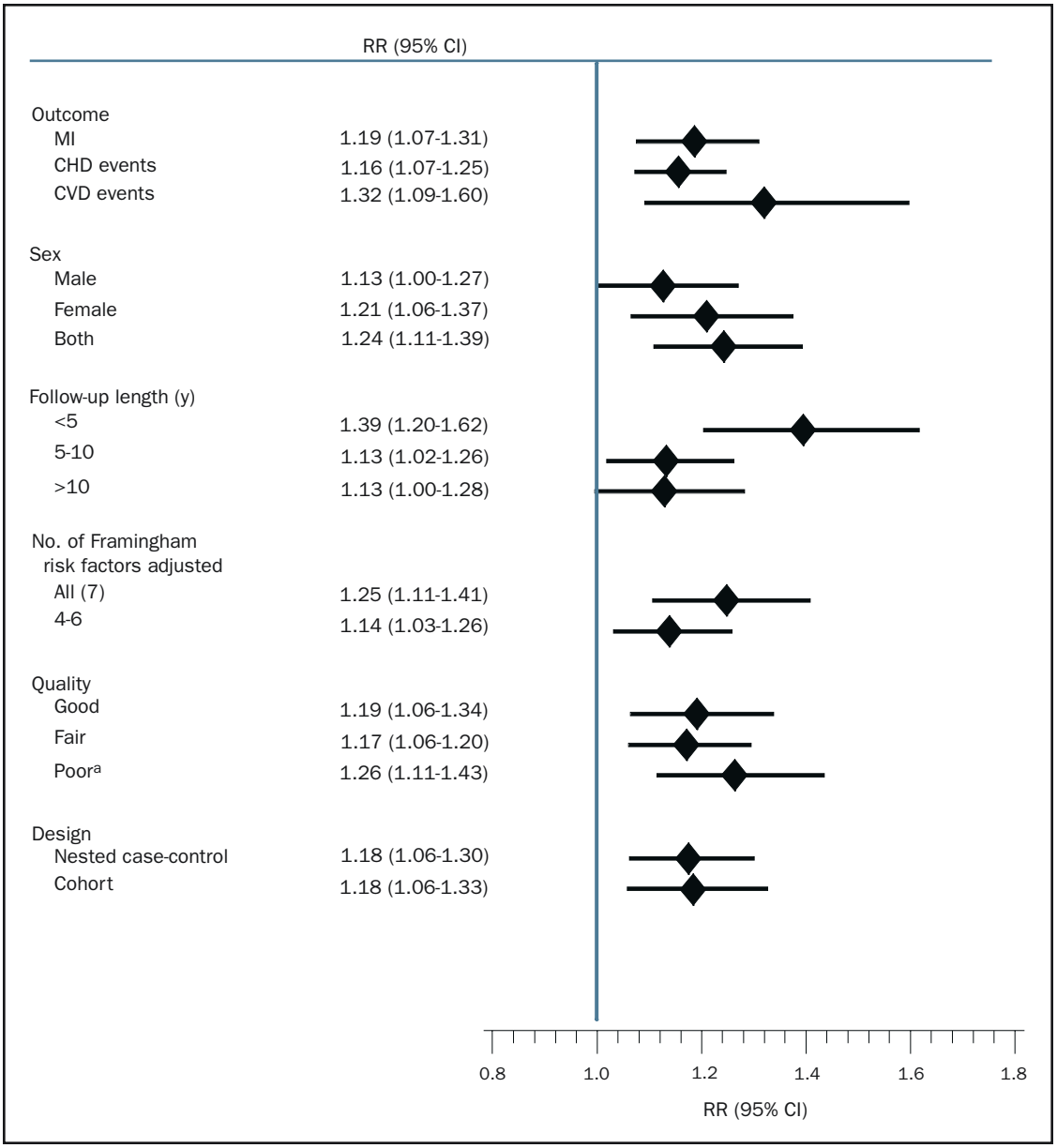

FIGURE 3. Risk ratio (RR) of coronary heart disease (CHD) associated with every 5-µmol/L increase of homocysteine level, stratified by selected characteristics. Myocardial infarction (MI) is a subgroup of CHD events. Risk factors are based on reference 2. CI = confidence interval; CVD = cardiovascular disease.
[a] Except for this analysis, all other subgroup analyses do not include poor studies.

others evaluated a combination of CHD outcomes. The resulting relative risk estimates among outcomes were sufficiently similar, however, to combine in meta-analysis. Studies also reported their findings variably, making it difficult to identify either a dose-response or threshold effect but relatively easy to identify that in most cohorts; those with higher homocysteine levels had worse CHD outcomes than those with lower levels. Another limitation is that the method of ascertainment of outcomes differed in each study. Some studies relied on population registries and others on self-report. We tried to account for this potential source of error in our quality ratings and minimize this problem by focusing only on fair or better quality studies in this review. We think it unlikely that bias in assigning the outcome is important in our findings because all included studies were prospective and homocysteine levels were measured long before the outcomes occurred.

This review has several strengths. We used only data from prospective cohort studies, most of which were large and population based. All participants in these studies had homocysteine levels measured at baseline and were followed up for the development of CHD. Although this does not allow us to determine whether homocysteine level is a causal risk factor or a marker of risk, it minimizes the problem of determining cause and effect that is associated with cross-sectional studies. The population-based nature of the cohorts substantially improves generalizability of the findings, which is of great importance when considering public health and prevention guidelines that are applied broadly in a population. Another strength of this review is that we rated the quality of all studies and eliminated those of poor quality with findings of questionable validity. However, even when these studies' findings were included in the review and meta-analysis, the association between homocysteine levels and CHD was similar.

Our results support the importance of completing ongoing trials that are evaluating whether the treatment of elevated homocysteine levels has a role in the *primary* prevention of CHD.[63,64] Despite widespread belief to the contrary, trials conducted to date have not determined whether treatment of elevated homocysteine levels before a person develops clinically recognized vascular disease is beneficial; no primary prevention trials have been completed. Two trials of tertiary prevention, the Heart Outcomes Prevention Evaluation 2 study[18] and the Norwegian Vitamin Trial,[17] found that vitamin B and folate supplementation did not reduce the risk of major cardiovascular events among patients with prevalent vascular disease (or diabetes), despite reducing homocysteine levels. However, these trials were of relatively short duration (<5 years) and involved the use of higher doses of folic acid than the dose necessary to reduce homocysteine levels. Furthermore, mean levels of homocysteine in these studies were in the normal range, and therapy was not directed at persons with elevated levels of homocysteine. Intriguingly, the Heart Outcomes Prevention Evaluation 2 study showed a significant decrease in stroke with a relative risk of 0.75 (95% CI, 0.59-0.97), supporting a possible role for folate therapy in modifying vascular disease. However, the results of the Norwegian Vitamin Trial conflict with this finding. A recent meta-analysis combining data from trials of folic acid supplementation among persons with established CHD (or stroke or end-stage renal disease) also showed no benefit but had the same limitations described herein.[65]

CONCLUSION

Elevated homocysteine levels independently and moderately increase the risk of developing CHD either in a causal manner or as a risk marker by approximately 20%. The prevalence of above-normal homocysteine levels in the United States has been shown to be higher than 5% to 10% in several population-based cohorts, even after widespread fortification of food with folic acid. Thus, if primary prevention treatment studies were to show benefit among persons with elevated homocysteine levels, many CHD deaths and events could potentially be prevented. Alternatively, if an elevated homocysteine level were to identify persons at higher risk than predicted by traditional risk factors, proven prevention strategies aimed at known risk factors might also substantially reduce the burden of CHD. The results of our analysis support the importance of data from trials currently under way that are evaluating the role of treating elevated homocysteine levels for primary prevention of CHD, as well as the allocation of resources to study the usefulness of including measurement of homocysteine levels in clinical risk assessment strategies to guide the intensity of conventional risk factor treatment.

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