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SYSTEMATIC REVIEWS AND META-ANALYSIS

Homocyst(e)ine and cardiovascular disease: a systematic review of the evidence with special emphasis on case-control studies and nested case-control studies

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Background Elevated concentrations of homocyst(e)ine are thought to increase the risk of vascular diseases including coronary heart disease and cerebrovascular disease.

Methods We searched MEDLINE (1966–1999), EMBASE (1974–1999), SciSearch (1974–1999), and Dissertation Abstracts (1999) for articles and theses about homocyst(e)ine concentration and coronary heart disease and cerebrovascular disease.

Results We included 57 publications (3 cohort studies, 12 nested case-control studies, 42 case-control studies) that reported results on 5518 people with coronary heart disease (11 068 control subjects) and 1817 people with cerebrovascular disease (4787 control subjects) in our analysis. For coronary heart disease, the summary odds ratios (OR) for a 5-μmol/l increase in homocyst(e)ine concentration were 1.06 (95% CI : 0.99–1.13) for 2 publications of cohort studies, 1.23 (95% CI : 1.07–1.41) for 10 publications of nested case-control studies, and 1.70 (95% CI : 1.50–1.93) for 26 publications of case-control studies. For cerebrovascular disease, the summary OR for a 5-μmol/l increase in homocyst(e)ine concentration were 1.10 (95% CI : 0.94–1.28) for 2 publications of cohort studies, 1.58 (95% CI : 1.35–1.85) for 5 publications of nested case-control studies, and 2.16 (95% CI : 1.65–2.82) for 17 publications of case-control studies.

Conclusions Prospective studies offer weaker support than case-control studies for an association between homocyst(e)ine concentration and cardiovascular disease. Although other lines of evidence support a role for homocyst(e)ine in the pathogenesis of cardiovascular disease, more information from prospective epidemiological studies or clinical trials is needed to clarify this role.

Keywords Homocyst(e)ine, meta-analysis, cardiovascular disease

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Homocyst(e)ine is a thiol-containing amino acid generated when the essential amino acid methionine is metabolized to cysteine. Homocystinuria, an inherited autosomal recessive disease, was first reported in 1962, from Ireland, where cystathionine β-synthase deficiency is particularly prevalent.[1] In 1969, McCully proposed that elevated homocyst(e)ine concentration could be a risk factor for cardiovascular disease.[2] In a meta-analysis of 27 studies published in 1995, Boushey and colleagues concluded

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that elevated homocyst(e)ine concentration was a risk factor for arteriosclerotic vascular disease.[3] These authors found that a rise in homocyst(e)ine concentration of 5 μmol/l was associated with odds ratios (OR) of 1.6 (95% CI : 1.4–1.7) for coronary artery disease and 1.8 (95% CI : 1.3–1.9) for cerebrovascular disease. Only three nested case-control studies were included in that review, and, thus, the conclusions were based largely on cross-sectional and case-control studies. Case-control studies, which are subject to a variety of biases, particularly selection bias, and cross-sectional studies are generally considered inferior to nested case-control studies and cohort studies in determining causation. Subsequently, several additional narrative reviews of homocyst(e)ine and cardiovascular disease have been published.[4–8] Because these reviews were not systematic and because new studies about homocyst(e)ine concentration as a risk factor for cardiovascular disease have been published, we updated the earlier meta-analysis. We produced separate risk estimates for cohort studies, nested case-control studies, and case-control studies and assessed the quality of the studies.

Methods

With the assistance of a librarian, we performed a literature search of three electronic databases using OVID version 2: MEDLINE (1966–1999), EMBASE (1974–1999), and SciSearch (1974–1999). For MEDLINE, we used the exploded terms homocysteine and cardiovascular disease. In EMBASE and SciSearch, we searched terms for homocyst(e)ine and cardiovascular disease that corresponded to the exploded terms in MEDLINE. In addition, we searched for doctoral theses using the Dissertation Abstracts database for 1999. We augmented these searches by examining references in papers and by searching our own files. We did not ask experts for references, and there were no language restrictions. We did not use unpublished studies.

We limited our analysis to case-control studies, nested case-control studies, and cohort studies of fatal and non-fatal coronary heart disease and cerebrovascular disease. We excluded case series of patients,[9,10] cross-sectional studies,[11–14] angiographic studies,[15–21] studies that did not provide results separately for patients with coronary heart disease and cerebrovascular disease,[22–28] studies of carotid artery stenosis or wall thickness measured by ultrasound, a study of coronary artery calcification,[29] a study of aortic atherosclerosis,[30] and studies of special populations such as patients on dialysis[31] and patients with systemic lupus erythematosus,[32] diabetes,[33,34] or cardiovascular disease.[35] Furthermore, we excluded studies which failed to report at least one of three types of data: mean concentrations and standard deviations of circulating homocyst(e)ine (plasma or serum) for case and control subjects, odds ratios (OR) or measures of relative risk for ⩾4 levels of homocyst(e)ine concentration, or reported OR or measures of relative risk for a defined change in homocyst(e)ine concentration.[17,36–42] All studies had to include a fasting or post-methionine loading homocyst(e)ine concentration.

Working in teams of two, six of us abstracted the studies and disagreements were resolved within the teams. When multiple papers from a single study had been published, we used the latest publication and supplemented it with data from the earlier publications. We did not contact authors to request additional data. We rated the quality of studies on five criteria; possible scores ranged from 0 to 10 (Table 1).[43–46]

Data Analysis

For studies that reported mean homocyst(e)ine concentration for a diseased group and a control group, we followed the methods used by Boushey and colleagues.[3] The pooled variance [S^2p] was calculated using the case and control group homocyst(e)ine variances weighted by their sample sizes. The slope was calculated by dividing the difference between the case and control means by S^2p. The log OR for a 5-μmol/l change in homocyst(e)ine concentration was calculated by multiplying the slope by 5 and the variance of the log OR by dividing the sum of inverse sample sizes by S^2p.

If a study reported standard errors, we estimated the standard deviations by multiplying the standard error by the square root of the sample size.[47,48] For two studies, we used the range of homocyst(e)ine concentration to estimate the standard deviations by dividing the range by 6.[49,50] For another study, we estimated the standard deviation by dividing by 2 the difference of the geometric mean and the geometric mean plus 2 standard deviations,[51] and for three others, we estimated the standard deviation from the 5th and 95th percentiles by taking the difference between the percentiles and dividing by 3.3.[52–54] For

Table 1 Quality scoring criteria

Criterion	Score
Study design	Cross-sectional study or angiographic or patient series = 1
	case-control study = 2
	nested case-control study = 3
Response rate	not given = 0
	1 ⩽75%
	2 >75%
Subject exclusion criteria	not given = 0
	criteria specified = 1
Types of controls	hospital or mixed = 1
	community = 2
Matching or adjustment for confounders	none = 0
	any confounder = 1
	age, smoking, hypertension, and cholesterol = 2

several studies, we assumed that medians or geometric means and standard deviations were equivalent to arithmetic means and standard deviations.[49,51–53,55,56] For one study, we calculated mean homocyst(e)ine concentrations for case patients from the raw data reported in the publication.[57] When studies had multiple control groups, we chose ones that were most likely to be population-based.

Some studies reported OR for disease at several (mean) concentrations of homocyst(e)ine, or 'doses'. When four or more doses are available, a response slope, which we refer to as a dose-response estimate, can be estimated in a linear weighted regression model.[58]

We calculated both fixed-effects and random-effects estimates. The study weights for the fixed-effects model were the inverse of the variances; random-effects weights were calculated by the DerSimonian method.[59] We stratified the analysis by: sex, study design, whether studies matched in the design phase, and quality of study.

For assessing heterogeneity among studies, we calculated both a weighted and unweighted χ^2 statistic.[60] Because these variances were not statistically equivalent, we calculated an unweighted χ^2.[61] Where results were statistically heterogeneous ($P < 0.10$), we checked for outliers.[62] In order to assess the influence of individual studies, we performed sensitivity analyses and show results with and without outliers. To examine the possibility that publication bias may have affected our results, we examined plots of the OR versus the standard errors of the studies.[63]

Results

Coronary heart disease

The 38 publications on coronary heart disease[47,51,53,55,64–97] included 5518 case subjects and 11 068 control subjects (Table 2). A single non-significant OR was <1.0.[70] The summary OR were 1.55 (95% CI : 1.40–1.71) for 36 publications of nested or case-control studies, 1.46 (95% CI : 1.32–1.62) for 23 publications of men and 1.92 (95% CI : 1.25–2.93) for 9 publications of women (Table 4).

Cohort studies

Two cohort studies of men (269 events among 3051 participants) have reported positive associations between homocyst(e)ine concentration and coronary heart disease.[64,65] The authors of the Zutphen Elderly Study reported an OR of 1.01 (95% CI : 0.993–1.069) per 1-μmol/l increase for incident events which is equivalent to an OR of 1.05 (95% CI : 0.96–1.15) per 5-μmol/l increase.[64] For the Caerphilly study, we calculated an OR of 1.07 (95% CI : 0.95–1.20).[65] The fixed effects summary OR was 1.06 (95% CI : 0.99–1.13). A third cohort study used cardiovascular disease, consisting of coronary heart disease and stroke, as its endpoint.[26]

Nested case-control studies

The 10 nested case-control studies of five different study populations had 1934 case subjects and 4285 control subjects (66–75). Six studies included only men[66,69–71,73,75] and four included men and women.[67,68,72,74] For eight studies that reported mean concentrations of homocyst(e)ine for case and control participants, the authors of four studies concluded that elevated homocyst(e)ine concentration increased the risk of coronary heart disease, while the authors of the other four failed to reject the null hypothesis of no association. We used the most recent data from three publications of the Physicians' Health Study (PHS)[69,98,99] to estimate an OR of 1.23 (95% CI : 1.06–1.41) for these eight studies. When we re-analysed the data using the earlier PHS data,[98] the OR was the same (OR = 1.23, 95% CI : 1.06–1.43). Among men, we estimated an OR of 1.19 (95% CI : 1.02–1.40).

For two additional nested case-control study, we were able to estimate an OR from dose-response data.[72,75] Adding these studies to the other eight nested case-control studies yielded a summary OR of 1.23 (95% CI : 1.07–1.41).

The only report that presented data separately for men and women found no significant association between homocyst(e)ine concentration and myocardial infarction for either sex.[67] In two other studies, sex did not modify the association between homocyst(e)ine concentration and coronary heart disease.[72,74] In addition, Arnesen *et al*. reported that the per 4-μmol/l change of homocyst(e)ine was 1.66 (95% CI : 0.67–4.12) for women compared with an adjusted relative risk 1.41 (95% CI : 1.16–1.71) for all subjects.[68]

We excluded one other nested case-control study of homocyst(e)ine concentration and cardiovascular disease among women in which coronary heart disease or stroke were combined.[28] A significant association between homocyst(e)ine concentration and cardiovascular disease was reported (OR = 1.24 per 5-μmol/l increase in homocyst(e)ine concentration).

Case-control studies

The 26 publications included 3315 case subjects and 4001 control subjects.[47,51,53,55,76–97] The summary OR were 1.70 (95% CI : 1.50–1.93) for all men and women, 1.63 (95% CI : 1.44–1.85) for men and 2.11 (95% CI : 1.30–3.42) for women (Table 4). For case-control studies of men and women in which the authors reported an OR for four or more categories of the homocyst(e)ine distribution, the summary OR was 1.45 (95% CI : 0.71–2.97).[51,82,83,85,87,93,100] The summary OR for studies that measured homocyst(e)ine after a post-methionine loading test was similar to that for studies that measured baseline homocyst(e)ine concentrations.

For studies that matched on age or other factors in selecting case and control subjects,[55,77,82,87,88,91–93,96] the summary OR was 1.49 (95% CI : 1.28–1.74); for studies that did not match the OR was 1.85 (95% CI : 1.54–2.23).

Generally, study results were not heterogeneous except for case-control studies among women. No single study accounted for the heterogeneity. Rather, there appeared to be two clusters of studies.

Study quality

Quality scores ranged from 6 to 9 for nested case-control studies and from 2 to 8 for case-control studies. After stratifying the case-control studies by a score of ⩾7 and <7, the summary OR was 1.46 (95% CI : 1.17–1.84) for the upper stratum[51,88,91,93,94] and 1.75 (95% CI : 1.52–2.00) for the lower stratum.

Cerebrovascular disease

The 24 publications of cerebrovascular disease[48–50,52,54,56,57,64,66,67,74,89,101–112] included 1817 case subjects and 4787 control subjects (Table 3). No study had a significant OR <1.0. The summary OR was 1.97 (95% CI : 1.61–2.40) for all nested case-control studies and case-control studies.

Table 2 Studies of homocyst(e)ine concentration and coronary heart disease

First author	Reference	Year of publication	Country	Study design	Gender[a]	Age[b] (mean or range)
Alfthan	67	1994	Finland	Nested case-control	M, F	40–64
Andersson	79	1991	Sweden	Case-control	M	50–69
Arnesen	68	1995	Norway	Nested case-control	Both	12–61
Blacher	47	1996	France	Case-control	Both	36–84
Bots	74	1999	Netherlands	Nested case-control	Both	>55
Chaco	95	1998	India	Case-control	Both	49.4/47.9
Dalery	80	1995	Canada	Case-control	M, F	<60
Dierkes	53	1998	Germany	Case-control	M	56.8/52.0
Evans	70	1997	United States	Nested case-control	M	35–57
Folsom	72	1998	United States	Nested case-control	Both	45–64
Freyburger	89	1997	France	Case-control	Both	50/35
Genest	78	1990	United States	Case-control	M	49
Gibelin	90	1997	France	Case-control	M, F, Both	47
Israelsson	77	1988	Sweden	Case-control	M	48–58
Israelsson	66	1993	Sweden	Nested case-control	M	53–65
Joubran	96	1998	Syria	Case-control	M	25–75
Kang	76	1986	United States	Case-control	Both	<70
Landgren	81	1995	Sweden	Case-control	M, F	~68
Loehrer	85	1996	Switzerland	Case-control	M, F, Both	51–98
Lolin	86	1996	Hong Kong	Case-control	M	<55
Ma	69	1996	United States	Nested case-control	M	40–84
Malinow	87	1996	France, Ireland	Case-control	M	25–64
Mendis	91	1997	Sri Lanka	Case-control	Both	35–73
Montalescot	92	1997	France	Case-control	Both	56
Pancharuniti	51	1994	United States	Case-control	M	30–50
Reis	82	1995	Portugal	Case-control	Both	<41–55
Robinson	83	1995	United States	Case-control	M, F, Both	62
Schwartz	93	1995	United States	Case-control	M, F, Both	18–44
Stehouwer	64	1999	Netherlands	Cohort	M	64–84
Ubbink	65	1998	United Kingdom	Cohort	M	50–64
Verhoef	71	1997	United States	Nested case-control	M	58.2
Verhoef	88	1997	Netherlands	Case-control	M	52.5
Verhoef	94	1996	United States	Case-control	M, F	57.9
Von Eckardstein	55	1994	Germany	Case-control	M	36–65
Wald	73	1998	United Kingdom	Nested case-control	M	35–64
Whincup	75	1999	United Kingdom	Nested case-control	M	40–59
Wu	84	1994	United States	Case-control	Both	56.2/47.5
Yoo	97	1999	Korea	Case-control	M	25–82

[a] M = male; F = female.

[b] Mean age listed; means for case and control subjects given respectively.

Cohort studies

Two cohort studies (263 events among 2780 participants) have examined the association between homocyst(e)ine concentration and incident cerebrovascular disease.[64,101] In the Zutphen Elderly Study, the authors reported that the risk for incident cerebrovascular disease increased by 1.01 (95% CI : 0.90–1.12) and for mortality from cerebrovascular disease by 1.04 (95% CI : 0.92–1.16) for a 5-μmol/l increase in homocyst(e)ine concentration.[64] Using the dose-response data from the Framingham study, we estimated that the risk for cerebrovascular disease increased by 1.17 (95% CI : 1.14–1.20) for a 5-μmol/l increase in homocyst(e)ine concentration.[101] The random-effects summary estimate of these two studies is 1.10 (95% CI : 0.94–1.28) per 5 μmol/l.

Nested case-control studies

Five publications included 316 case subjects and 1250 control subjects.[52,66,67,74,102] The summary OR was 1.58 (95% CI : 1.35–1.85). Among men, the summary OR was 1.56 (95% CI : 1.30–1.88). The fixed-effects OR among women for a single study was 1.10 (95% CI : 0.98–1.24).[67]

Case-control studies

Seventeen publications included 1121 case subjects and 902 control subjects.[48–50,54,56,57,89,103–112] The summary OR was

Table 3 Studies of homocyst(e)ine concentration and cerebrovascular disease

First author	Reference	Year of publication	Country	Study design	Gender[a]	Age[b] (mean or range)
Alfthan	67	1994	Finland	Nested case-control	M, F	40–64
Araki	103	1989	Japan	Case-control	Both	39–79
Boers	57	1985	Holland	Case-control	M, F	<50
Bostom	101	1999	United States	Cohort	Both	59–91
Bots	74	1999	Netherlands	Nested case-control	Both	>55
Brattstrom	48	1984	Sweden	Case-control	Both	35–63
Brattstrom	104	1990	Sweden	Case-control	Both	41.1, 51.9/52.2
Brattstrom	106	1992	Sweden	Case-control	Both	38–90
Candito	109	1997	France	Case-control	Both	22–55
Coull	105	1989	United States	Case-control	Both	60–67
Delport	50	1997	Australia	Case-control	Both	60
Deulofeu	107	1996	Spain	Case-control	Both, M, F	20–85
Evers	110	1997	Germany	Case-control	Both	59
Freyburger	89	1997	France	Case-control	Both	44/35
Israelsson	66	1993	Sweden	Nested case-control	M	53–65
Kristensen	112	1999	Sweden	Case-control	Both	18–44
Lindgren	49	1995	Sweden	Case-control	Both	⩾50
Markus	56	1997	United States	Case-control	Both	65.7/65.4
Perry	52	1995	United Kingdom	Nested case-control	M	40–59
Reis	108	1995	Portugal	Case-control	Both	<55
Stehouwer	64	1999	Netherlands	Cohort	M	64–84
Verhoef	102	1994	United States	Nested case-control	M	40–84
Vila	111	1998	Spain	Case-control	Both	59.1/56.7
Yoo	54	1998	Korea	Case-control	M	39–82

[a] M = male; F = female.

[b] Mean age listed; means for case and control subjects given respectively.

2.16 (95% CI : 1.65–2.82). Because these studies appeared to be heterogeneous, we removed a single study[49] that had the largest homogeneity statistic. Consequently, the remaining studies were no longer heterogeneous and the summary OR changed to 2.25 (95% CI : 1.76–2.87). The OR were 1.95 (95% CI : 1.33–2.85) for men,[54,57,106,107] and 1.56 (95% CI : 1.09–2.24) for women.[57,106,107]

For studies that matched on age or other factors in selecting case and control subjects,[49,50,103,108,109] the summary OR was 2.49 (95% CI : 1.05–5.91) and 2.06 (95% CI : 0.92–4.63) after an outlier was eliminated.[103] For studies that did not match, the OR was 2.06 (95% CI : 1.60–2.66).

Study quality

Quality scores for case-control studies ranged from 2 to 7 with only a single study achieving the top score.

Publication bias

Funnel plots of the study effect size plotted against the study's weight for coronary heart disease and cerebrovascular disease suggested that we had not selectively omitted negative studies. However, funnel plot asymmetry was present for case-control studies of coronary heart disease (intercept = 3.1; $P = 0.0001$) as well as cerebrovascular disease (intercept = 3.3; $P = 0.0001$) but not for nested case-control studies of coronary heart disease.[113]

Discussion

In the most comprehensive meta-analysis to date, we have reviewed 57 publications that explored the relationship between homocyst(e)ine concentration and coronary heart disease or cerebrovascular disease. For coronary heart disease studies, we calculated summary OR for a 5-μmol/l increase in homocyst(e)ine of 1.06, 1.23, and 1.70 for cohort studies, nested case-control studies, and case-control studies, respectively. For cerebrovascular disease studies, these summary OR were 1.10, 1.58, and 2.16 for cohort studies, nested case-control studies, and case-control studies, respectively. Thus, the prospective studies, which are generally considered to have a stronger study design than case-control studies, found a weak but significant association between homocyst(e)ine concentration and coronary heart disease risk but a more robust association between homocyst(e)ine and cerebrovascular disease.

Heterogeneity and bias

We calculated both weighted and unweighted χ^2 values for an assessment of heterogeneity. The weighted χ^2 requires equality of intra-study variances. This requirement will often not be met in meta-analyses. Additionally, the weighted test may yield statistically significant results even with relative homogeneous means, if within-study variances are underestimated. This can happen when the true homocyst(e)ine variability

Table 4 Summary estimates of risk for coronary heart disease or cerebrovascular disease associated with changes in homocyst(e)ine concentration

				Odds ratios per 5-µmol/l increase in homocyst(e)ine							
				Fixed-effects model		Random-effects model		Weighted		Unweighted	
Study design[a]		Gender	No. of odds ratios	Mean	95% CI	Mean	95% CI	Homogeneity χ^2	*P*-value for χ^2	Homogeneity χ^2	*P*-value for χ^2
Coronary outcomes											
Baseline total homocyst(e)ine											
CC & NCC		Total	39	1.33	1.29–1.38	1.55	1.40–1.71	244.05	<0.001	37.72	0.48
CC & NCC	Total (outlier removed)		38	1.32	1.28–1.37	1.49	1.36–1.63	192.13	<0.001	22.27	0.97
NCC only		Total	10	1.25	1.16–1.36	1.23	1.06–1.41	25.59	0.002	1.76	0.99
CC only		Total	29	1.35	1.30–1.41	1.70	1.50–1.93	215.32	<0.001	33.30	0.22
CC only	Total (outlier removed)		28	1.34	1.29–1.40	1.61	1.44–1.80	164.08	<0.001	18.98	0.87
Cohort		Males	2	1.06	0.99–1.13	NA		0.06	0.804	0.003	0.959
CC & NCC		Males	25	1.39	1.32–1.45	1.46	1.32–1.62	93.41	<0.001	9.30	0.99
NCC only		Males	8	1.23	1.13–1.33	1.19	1.02–1.40	21.93	0.003	1.50	0.98
CC only		Males	18	1.48	1.41–1.56	1.63	1.44–1.85	78.38	<0.001	5.80	0.99
CC & NCC		Females	9	1.17	1.09–1.27	1.92	1.25–2.93	60.80	<0.001	75.80	<0.001
NCC only		Females	1	1.00	0.91–1.10	NA		NA			
CC only		Females	8	1.18	1.09–1.27	2.11	1.30–3.42	60.72	<0.001	64.47	<0.001
Dose-response-all[b]		Total	12	1.17	1.04–1.31	1.29	0.73–2.27	209.44	<0.001	1.26	0.99
Dose-response-NCC only		Total	4	1.02	0.88–1.20	1.07	0.52–2.22	36.95	<0.001	0.25	0.99
Dose-response-CC only		Total	8	1.36	1.14–1.63	1.45	0.71–2.97	90.54	<0.001	0.30	0.99
Post-methionine load											
CC only		Total	6	1.21	1.12–1.31	1.58	1.30–1.92	20.63	0.002	6.29	0.28
CC only	Total (outlier removed)		5	1.18	1.09–1.28	1.25	1.07–1.47	9.34	0.053	2.28	0.68
Cerebral outcomes											
Baseline total homocyst(e)ine											
Cohort		Total	2	1.17	1.16–1.17	1.12	1.01–1.25	7.30	0.007	0.15	0.701
CC & NCC		Total	26	1.46	1.36–1.57	1.97	1.61–2.40	147.24	<0.001	31.16	0.19
CC & NCC	Total (outlier removed)		25	1.78	1.63–1.94	1.99	1.66–2.38	83.21	<0.001	14.89	0.93
NCC only		Total	6	1.60	1.40–1.83	1.58	1.35–1.85	5.90	0.104	0.58	0.99
CC only		Total	20	1.41	1.30–1.53	2.16	1.65–2.82	138.83	<0.001	35.11	0.01
CC & NCC		Males	8	1.60	1.39–1.84	1.66	1.34–2.07	14.08	<0.001	2.89	0.89
NCC only		Males	5	1.60	1.39–1.83	1.56	1.30–1.88	5.85	0.063	0.57	0.99
CC only		Males	4	1.84	1.50–2.27	1.95	1.33–2.85	8.23	0.093	1.48	0.70
CC & NCC		Females	3	1.59	1.15–2.20	NA		3.98	0.150	1.13	0.57
NCC only		Females	1	1.10	0.98–1.24	NA		NA	NA		
CC only		Females	3	1.56	1.09–2.24	NA		NA	NA		
Dose-response		Total	1	1.15	1.05–1.30	NA		NA	NA		
Post-methionine load											
CC only		Total	5	1.60	1.37–1.88	NA		5.57	0.233	0.64	0.96

[a] CC = case-control studies; NCC = nested case-control studies.

[b] Dose-response refers to the calculation of the odds ratio from studies that reported odds ratios for four or more categories of the momocyst(e)ine distribution.

NA = Not applicable: single study or random effects model not applicable.

among cases or controls is underestimated from an apparently very homogeneous small group of subjects. Because of these considerations, we considered that the unweighted χ^2 statistic, which showed little heterogeneity among studies except for case-control studies among women and case-control studies of cerebrovascular disease among men and women, might be preferable for this analysis. We were unable to determine the reasons for the apparent heterogeneity for case-control studies of women. For case-control studies of cerebrovascular disease, eliminating a single study resolved the apparent heterogeneity. This study had the largest number of case subjects and was one of only two studies that found a lower mean or median homocyst(e)ine concentration among case subjects than control subjects.

Although the funnel plots did not suggest to us that publication bias was evident, funnel plot asymmetry was present among case-control studies of coronary heart disease and cerebrovascular disease.[113] For both sets of studies, the intercepts

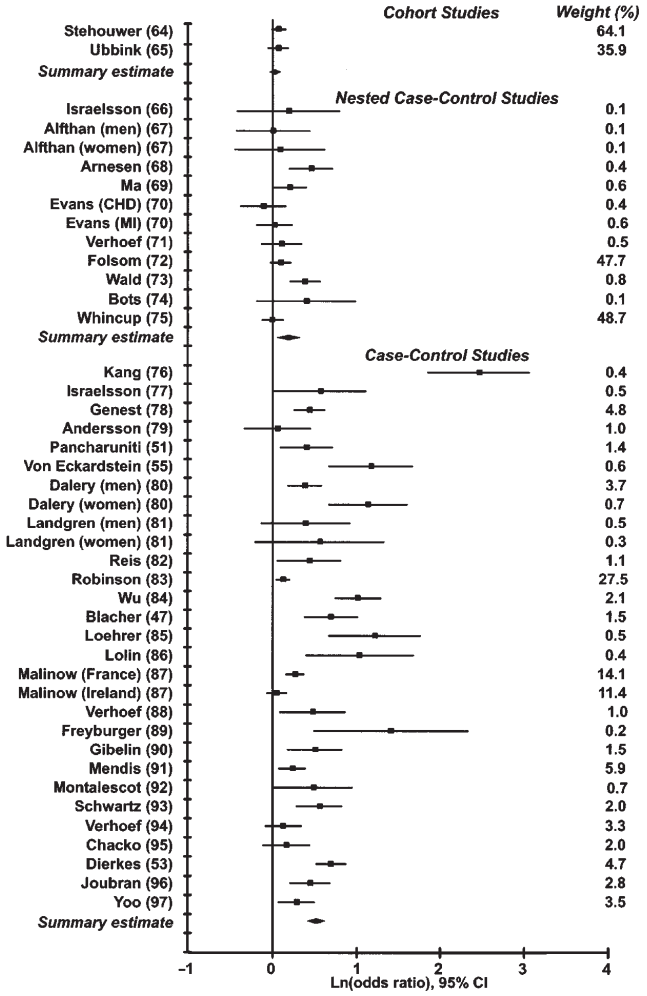

Figure 1 Estimated odds ratios per 5-μmol/l change in homocyst(e)ine concentration and coronary heart disease by individual cohort studies, nested case-control studies, and case-control studies. Odds ratios are plotted in order of year of publication and, within year of publication, according to alphabetical order of first author's name. If authors of studies that included men and women reported results for the two sexes combined, a single odds ratio representing the combined sample was graphed. Otherwise, if no combined results were reported, sex-specific odds ratios were graphed

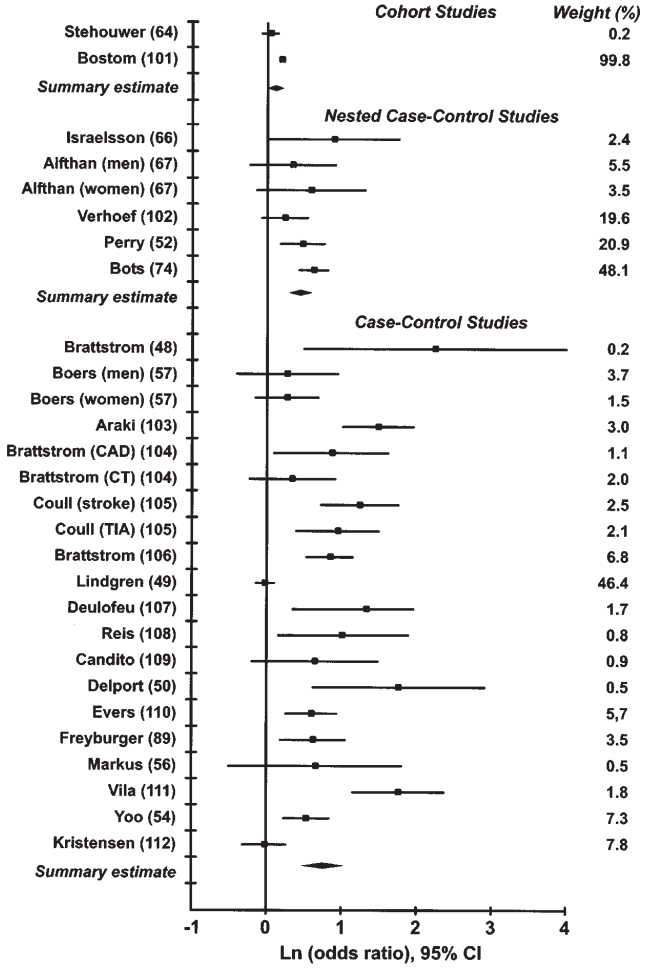

Figure 2 Estimated odds ratios per 5-μmol/l change in homocyst(e)ine concentration and cerebrovascular disease by individual cohort studies, nested case-control studies, and case-control studies. Odds ratios are plotted in order of year of publication and, within year of publication, according to alphabetical order of first author's name. If authors of studies that included men and women reported results for the two sexes combined, a single odds ratio representing the combined sample was graphed. Otherwise, if no combined results were reported, sex-specific odds ratios were graphed

for regression equations were positive and significant. In both instances, the slope was negative but not significant. Regardless of sample size threshold, funnel plot asymmetry, which can be caused by several sources of asymmetry, including selection bias, true heterogeneity, data irregularities, artefacts, or chance, persisted.[113] Although the unweighted χ^2 statistic we used to test for heterogeneity among OR did not indicate concerns about the presence of heterogeneity, the funnel plot asymmetry suggested otherwise. Our attempts to find possible sources of heterogeneity were not successful, however.

Excluded studies

Although we excluded various studies because of our study entry criteria, they do contain important information. Four cross-sectional studies[11–14] and seven angiographic studies[15–21] generally reported significant positive associations between homocyst(e)ine concentrations and cardiovascular disease. Five studies found a significant positive relationship between homocyst(e)ine concentration and carotid artery stenosis or intima or media thickness, an outcome that failed to meet our endpoint specification.[114–118]

In addition, the majority of case-control studies we excluded because the authors did not report their data in a format we could use reported a significant association between homocyst(e)ine concentration and coronary heart disease or cerebrovascular disease.[36–40,42] These studies had 600 subjects with coronary heart disease, 325 subjects with cerebrovascular disease, and 778 control subjects. Although the reported or calculated OR for these studies tended to be higher than the summary OR for the case-control studies we included, adding the five studies would not have materially affected our conclusions.

Results of prospective versus retrospective studies

The results from cohort and nested case-control studies differed substantially from those for case-control studies. Generally, the study quality of the nested case-control studies was superior to that of case-control studies. Odds ratios of case-control studies of coronary heart disease were lower for higher quality studies than for lower quality ones. Data show that homocyst(e)ine concentrations decline during an acute cardiovascular event and rise after the event.[49,81,85,119] How concentrations measured after an acute event compare with those before the acute event remains unknown, however. Additionally, some data suggest that endothelial cells injured by the atherosclerotic process may leak homocyst(e)ine into the circulation, resulting in an elevated homocyst(e)ine concentration.[120] Thus, the timing of blood sample collection with respect to a cardiovascular disease event may affect the results.

A finding that homocyst(e)ine concentrations in stored blood specimens were unstable over time could explain why short-term studies would produce significant associations and longer-term studies would not. Verhoef and Stampfer thought this was an unlikely explanation but did not dismiss it entirely.[121] Furthermore, freeze-thaw cycles are not thought to affect homocyst(e)ine concentrations.[72] Nested case-control studies, which were not designed specifically to test the hypothesis that homocyst(e)ine concentration is a risk factor for cardiovascular disease, may not have followed proper blood collection and processing procedures, possibly narrowing any differences in homocyst(e)ine concentration between cases and controls and biasing the OR towards the null hypothesis. Fasting status does not appear to account for observed differences.[122]

Laboratory methods

Reporting of laboratory methods was inadequate in many studies. Often, authors did not describe the blood collection and processing methods adequately. For example, if samples are not held on ice, erythrocytes will continue to produce homocyst(e)ine increasing its concentration before centrifugation.[123]

Few studies address issues of quality control of the homocyst(e)ine assay. Preliminary data from the Centers for Disease Control and Prevention on 14 laboratories performing this assay on reference materials indicate a between-laboratory coefficient of variation of 12.1% to 13.3% in the normal range.[124] For one reference material with a mean concentration of 11.1 μmol/l, the reported values ranged from 8.3 to 14 μmol/l. In the future, issues of quality control and laboratory standardization will need to be addressed.

Challenges

Combining the studies quantitatively proved difficult. Authors tended to report their results in various ways, used different cutoff values for establishing normal ranges, did not always report the boundaries of these homocyst(e)ine quantiles, and were unlikely to report an OR per μmol/l change of homocyst(e)ine concentration. Thus, the OR that we calculated from the reported homocyst(e)ine concentration means were largely based on unadjusted data, since most authors reported unadjusted means only. Because homocyst(e)ine concentrations increase with age, and because in a number of these studies the control subjects were younger than case subjects, some of the reported differences in mean homocyst(e)ine concentrations may have been attributable to age. Accordingly, the summary OR produced may have overestimated the association between homocyst(e)ine concentration and cardiovascular disease.

Attempting to incorporate study quality into a meta-analysis is a controversial subject.[45] In general, studies with higher quality scores reported a smaller effect size than studies with lower quality scores. Furthermore, studies of coronary heart disease, but not cerebrovascular disease, that had matched on one or more variables in the design phase produced a lower summary OR than studies that had failed to match.

If homocyst(e)ine is indeed a risk factor for cardiovascular disease, the form of the relationship (linear, curvilinear, or threshold) needs to be established. To date, no accepted optimal homocyst(e)ine concentration in humans has been defined based on epidemiological or other data. Such data are needed to formulate treatment and screening guidelines for health professionals and for public policy, such as the setting of objectives for population means and distributions. One way to define the form of the relation between homocyst(e)ine concentration and cardiovascular disease and to define an optimal upper limit would be to pool the data from the various nested case-control studies.

Recommendations

To facilitate the performance and review of meta-analyses in the future, investigators should report detailed blood collection and processing methods, information about quality control practices, the time interval from illness event to the blood draw as well as the interval from the blood draw to analysis, and both crude and adjusted homocyst(e)ine means and standard deviations for cases and controls. Furthermore, investigators should report the regression coefficient and standard error or the OR and confidence limits per unit or multiple unit change of homocyst(e)ine concentration, examine the form of the exposure-disease relationship by checking for non-linearity, and present adjusted risk estimates in addition to crude or age-adjusted estimates. The optimal set of potential confounders is not yet clear but should include age at a minimum. Also, because risk estimates may differ for different outcomes, the results should be presented separately for coronary heart disease, stroke and other manifestations of cardiovascular disease.

Conclusions

Homocyst(e)ine concentration is only weakly related to coronary heart disease and somewhat more strongly related to cerebrovascular disease. Additional prospective studies or clinical trials may help to clarify the relation between homocyst(e)ine concentration and risk of cardiovascular disease with care taken to include women and minority populations. Prospective studies suggest that the population attributable fraction of hyperhomocyst(e)inaemia may be smaller than previously thought and may be smaller than that of other proven, highly prevalent, modifiable risk factors for cardiovascular disease such as smoking, hypertension, hypercholesterolaemia, sedentary lifestyle, and overweight. At present, it is premature to formulate public health recommendations on recommended homocyst(e)ine concentrations, screening policies, and prevention measures in the general population.

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Commentary: An updated review of the published studies of homocysteine and cardiovascular disease

Robert Clarke

Over the last decade, evidence has accumulated that elevated plasma total homocysteine concentrations are associated with an increased risk of atherosclerotic and thromboembolic events.[1–3] Plasma homocysteine concentrations reflect genetic and environmental factors including diet. Vitamin supplementation with folic acid and vitamin B-12 achieves substantial reductions in blood homocysteine concentrations.[4] Several large-scale clinical trials are currently under way to assess whether vitamin supplementation to lower homocysteine concentrations can reduce vascular risk.[5] Accurate estimates of the likely strength of association of homocysteine with cardiovascular disease are necessary for the rational design and interpretation of the results of such trials. There have been several qualitative and quantitative reviews on homocysteine and risk of cardiovascular disease and each has been informative at the time of their separate publication.[1–3] Such systematic reviews can avoid selective biases, minimize random error and provide summary measures of effect based on the totality of available published data. The review by Ford *et al*.[6] in this issue of the *International Journal of Epidemiology* set out to provide an updated summary of the published evidence from observational studies on plasma total homocysteine and risk of cardiovascular disease. They abstracted from each publication either the reported odds ratio or relative risk for a change in homocysteine concentration; or the odds ratio or relative risk for more than four levels of homocysteine concentration; or the mean and standard deviation of homocysteine concentrations in cases and controls. They used these data to calculate the log odds ratio for a 5-μmol/l increase in homocysteine concentration and a pooled variance from the case and control group variance weighted by their sample sizes. One important study (COMAC case-control study) has been excluded, but the results of this review are unlikely to be materially altered by this exclusion. The most striking finding of the meta-analysis is the marked heterogeneity between the results of studies of different designs. The odds ratio of coronary heart disease for a 5-μmol/l increase in homocysteine concentration was 1.06 (95% CI : 0.99–1.13) for 2 cohort studies, 1.23 (95% CI : 1.07–1.41) for 10 nested case-control studies and 1.70 (95% CI : 1.50–1.93) for 26 case-control studies (Figure 1).

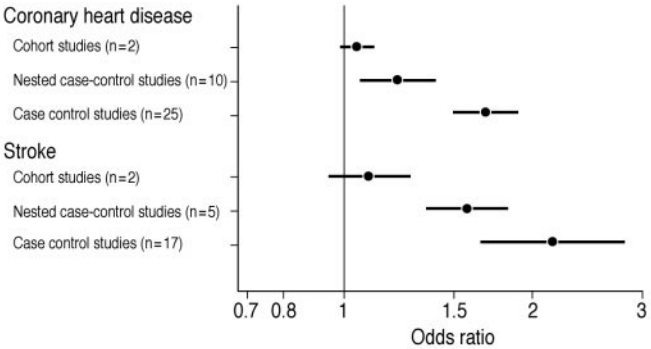

Figure 1 Association of a 5-μmol/l increase in homocysteine concentration with the probability of coronary heart disease and stroke. Meta-analysis of observational studies stratified by study design. Adapted from Ford *et al*.[6]

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The strength of association and heterogeneity between the results of studies of homocysteine and risk of stroke was even more extreme than for coronary heart disease. The odds ratio for a 5-μmol/l increase in homocysteine concentration for stroke was 1.10 (95% CI : 0.94–1.28) for 2 cohort studies, 1.58 (95% CI : 1.35–1.85) for 5 nested case-control studies and 2.16 (95% CI : 1.65–2.82) for 17 case-control studies (Figure 1). This updated summary of a large number of published studies illustrates the strength and limitations of systematic reviews of published data from observational studies. This review highlighted the heterogeneity between the results of individual studies, but was unable to explain the reasons for such heterogeneity. The review was unable to distinguish the extent to which the discrepant results of individual studies were due to confounding (due to differences in other aspects of lifestyle or cardiovascular risk factors) or bias (due to the effects of underlying disease or effects of other systematic differences) on homocysteine concentrations.

An individual patient data meta-analysis of the observational studies of homocysteine and cardiovascular disease is currently being co-ordinated by the Clinical Trial Service Unit to address these and other related questions on the age- and sex-specific relevance of homocysteine with risk of heart disease and stroke. Individual patient data overviews, which involve central data collection, validation and re-analysis of the data from individual studies on behalf of the collaborative group, can address issues in a way that it is not possible to do in a meta-analysis of published studies. Individual patient data meta-analysis can explore reasons for heterogeneity such as differential effects of prior vascular disease, age at screening, age at event and interval between screening and event. Moreover, individual patient data overviews can assess the effects of confounding by known risk factors. Individual patient overviews often involve collection of additional information to address particular questions such as the impact of bias, which is required to interpret the results of the overview. The present review illustrates that both types of systematic reviews may be informative in particular circumstances. The unexplained heterogeneity between the results of different study types suggests the results of the present review should be interpreted with caution.

Accurate assessment of the true strength of risk associations for differences in homocysteine concentrations after controlling for bias and confounding are necessary for prediction of the likely treatment effects in clinical trials. The results of clinical trials of homocysteine lowering therapy are necessary to assess treatment effects particularly where causal associations are uncertain and where residual confounding cannot be fully excluded and where risk associations are not likely to be fully reversible. The results of these large-scale trials (and possibly a further meta-analysis of post-publication results of individual trials) are required before formulating public health recommendations on screening for homocysteine concentrations or advocating fortification of foods with folic acid to reduce cardiovascular risk.

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