Plasma Total Homocysteine and its Relationship with Cardiovascular Disease

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Abstract: *Aims*: The specific aim of this review was to compile the first systematic review of systematic reviews and meta-analyses from a range of studies that evaluates the evidence that elevated homocysteine may be a risk factor for CVD.

Data Synthesis: 379 entries were identified by initial screening using set criteria revealing eleven meta-analyses, one systematic review, two systematic reviews/meta-analyses and ten other studies, between 1994 and 2013.These studies compared homocysteine levels and its relationship with twelve different types of CVD chronic conditions. Final methodological quality assessment was conducted independently using the instrument AMSTAR for the systematic reviews and meta-analyses. The remaining studies were assessed using data extraction tools from JBI QARI, Appendix 2 & 4 packages.

Conclusions: From the selected studies, 82.8% of the CVD conditions demonstrated that epidemiologic and clinical data strongly indicated that elevated homocysteine levels is a risk factor for primary CVD. 71.4% of the CVD conditions demonstrated that plasma tHcy can be employed as an independent biomarker. Despite 46.2% of the CVD conditions finding that reducing plasma tHcy lowers the risk of many CVD events, it remains unclear whether the reduction in plasma tHcy will reduce the risk of some CVD events; it is therefore considered prudent to take precautionary measures to aim for normal levels of homocysteine to avoid the risk of developing or exacerbating CVD. Moreover, it was shown that levels of homocysteine can be profoundly affected by diet, supplementation and lifestyle.

The present study will help to clarify the present scientific understanding of this subject.

Keywords: Hyperhomocysteinemia, cardiovascular disease, metabolism, diet, lifestyle.

INTRODUCTION

Many published studies have addressed the question, 'is elevated plasma tHcy related to CVD'? The present study reviews the research already undertaken and forms the first systematic review of systematic reviews and meta-analyses on this topic and aims to address the following questions:

1) Are elevated levels of plasma tHcy a risk factor for primary CVD?

2) Are elevated homocysteine levels a major independent biomarker for the risk of developing CVD?

3) Does normalising plasma tHcy reduce the risk of CVD?

Homocysteine is an intermediate product of the one-carbon metabolism. It is formed during the metabolism of methionine in the methionine cycle. Methionine from the diet is activated by adenosine triphosphate and used either for protein synthesis or the formation of SAM, which contains a very reactive methyl group. SAM is demethylated to S-adenosylhomocysteine. In the transsulfuration pathway, homocysteine is converted to cystathionine by the enzyme cystathionine beta-synthase which requires the essential cofactor vitamin B6. Homocysteine can also be remethylated through the folate cycle which is the major route for remethylation. In this route homocysteine is recycled to methionine in a reaction catalyzed by the enzyme methionine synthase which requires the essential vitamin B12, but only in its methylcobalamin form [1], see Figure **1**.

Deficiencies in vitamin B6, B12, folate and betaine usually results in elevated plasma homocysteine.

The normal range of plasma tHcy level has been defined by Ravaglia *et al.* [2] as 5 to 15 μmol/L, and HHCY at a plasma tHcy level of >15μmol/L. Refsum *et al.* [3] defined 15 μmol/L to 30 μmol/L level of plasma tHcy as 'Moderate HHCY', 30 μmol/L to 100 μmol/L level of plasma tHcy as 'Intermediate HHCY' and > 100 μmol/L level of plasma tHcy as 'Severe HHCY'.

RESEARCH FINDINGS

There is often a cluster of factors leading to elevated plasma tHcy levels. Many of these factors cause a change in plasma tHcy concentrations by

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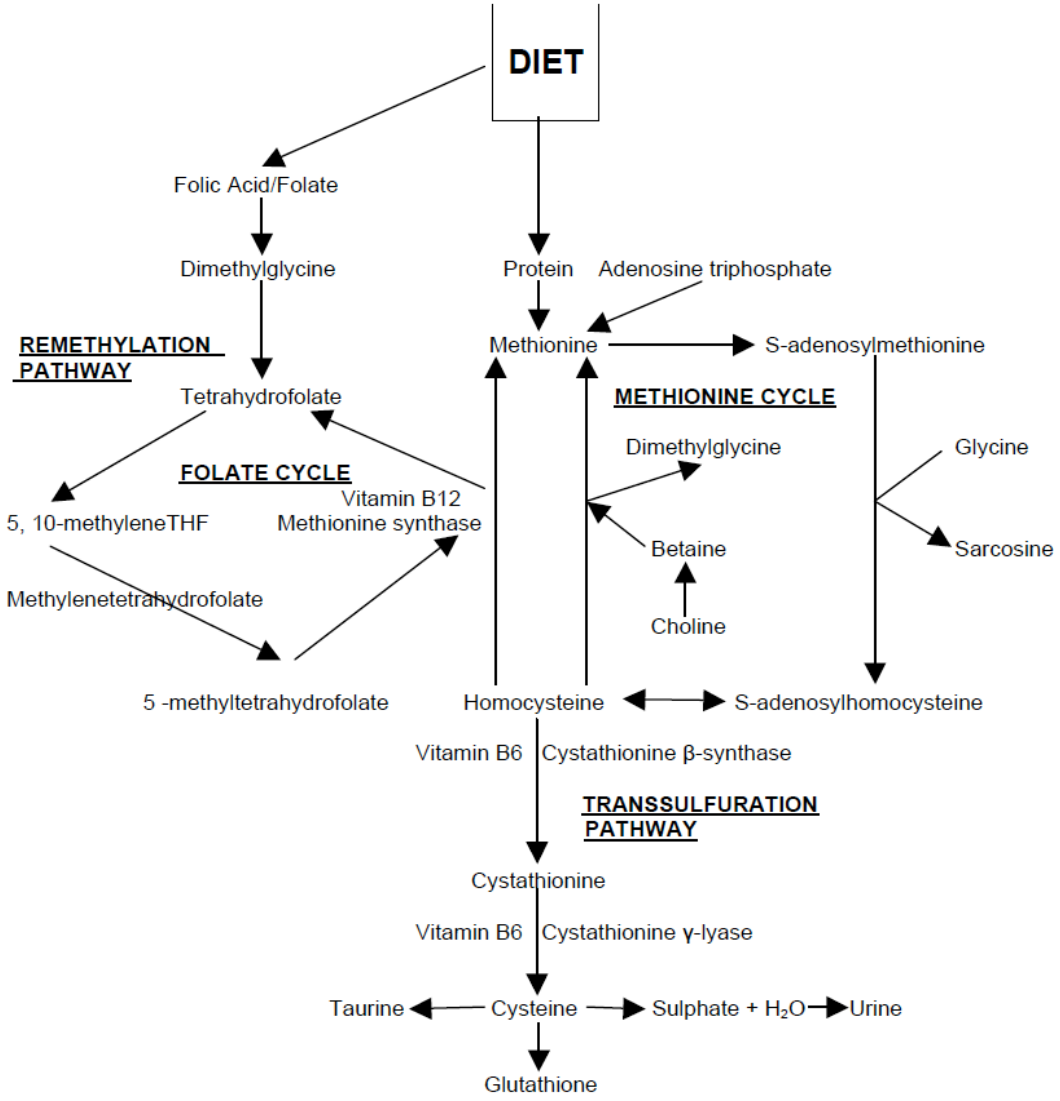

Figure 1: The methionine cycle, remethylation pathway of homocysteine to methionine *via* the folate cycle, the transsulfuration pathway, and the betaine reaction.

altering the function or plasma concentrations of the B vitamins, and/or by influencing renal function or more rarely by influencing enzyme activities [3].

Homocystinuria and Defective MTHFR Enzyme

Homocystinuria is a rare inborn autosomal recessive genetic disorder, characterised by massively increased levels of plasma tHcy, leading to severe HHCY [4]. According to Wilcken and Wilcken, [5] lowering elevated plasma tHcy in patients with homocystinuria (severe HHCY) due to CBS deficiency, greatly reduces cardiovascular risk. Much more common than homocystinuria are MTHFR mutations, such as MTHFR A1298C with the most common being the MTHFR C677T mutation. Related research has concluded that high tHcy concentration is typically associated with homozygosity for the 677C → T variant of MTHFR which occurs only with poor vitamin B2 status [6].

Age and Gender

According to Jacques *et al.* [7] levels of plasma tHcy rise with age, for males from a mean value of 6.84 µmol/L at the age of 12 to 15, rising to 12.4 µmol/L at the age of 80+.

Over the same age range females plasma tHcy levels rise from a mean value of 6.12 µmol/L to 11.44 µmol/L.

Raised Plasma Total Homocysteine Level During Female Menstruation

During the menstrual cycle, mean homocysteine levels increase from an average of 7.8 µmol/L in the

luteal phase to 8.9 µmol/L in the follicular phase, providing evidence that there are significant differences in plasma tHcy levels during the menstrual cycle [8].

Raised Plasma tHcy Level of Vegetarians

These individuals may be susceptible to elevated homocysteine, due to the lack of dietary animal produce and are potentially at risk to develop vitamin B12 deficiency homocysteine related chronic conditions [9]. Obersby *et al.* [9] recently demonstrated that a negative exponential relationship of the form $Y=Ae^{-BX}$ exists between plasma tHcy and serum vitamin B12 for omnivores, LV-LOV's and vegans.

Protein and B-Vitamins

Excessive animal protein in the diet can raise homocysteine levels. Inadequate intake of B-vitamins results in reduced enzyme function with the potential to elevate plasma tHcy levels [10]. In the case of folate deficiency, plasma homocysteine may be increased 8 to 10 fold together with a 2.5 fold increase for vitamin B12 deficiency, but is less affected by vitamin B6 deficiency (except for heterozygous or homozygous of CBS deficiency) [11]. In 1996 the FDA issued a regulation requesting that all enriched grain products contain 140 µg of folic acid per 100g. A subsequent study has confirmed that folic acid fortification has had a substantial effect on plasma folate and homocysteine concentrations [12]. In a population-based sample of middle-aged and older adults, plasma folate levels increased whilst homocysteine levels reduced by approximately 50% among those who did not take other supplements, resulting in a reduction of CVD in the USA by 2.9% per year and in Canada by 5.4% per year (1990 to 2002) [12].

Salt

Animal research has shown that a combination of high salt and high homocysteine levels cause the lining of the arteries to become more severely damaged, which is likely to increase susceptibility to heart disease [13].

Saturated Fat

It has been demonstrated [14] that high dietary levels of saturated fat raise homocysteine levels. Studies conducted on individuals consuming diets containing a range of saturated fat levels; show that low fat diets decreased homocysteine levels by 14%, while high fat diets raised homocysteine levels by 10% [14].

Betaine

Dietary betaine is important because of its role in the donation of methyl groups to homocysteine to form methionine, hence helping to maintain homocysteine levels within normal limits. Betaine can be synthesized in the body from choline [15] (see Figure **1**).

Tea and Coffee

A study carried out by Olthof [16] demonstrated that eight cups of coffee or black tea, (which contains high doses of chlorogenic acid and caffeine) per day, taken for a week raises homocysteine levels by 11% and 12% respectively.

Alcohol

Bleich *et al.* [17] demonstrated that plasma tHcy levels are raised by alcohol and the type of drink consumed. He gave 30g/day of alcohol to three groups of social drinkers (n=15) in the form of beer, red wine and spirits, mineral water was given to non-drinkers (n=15) for six weeks. The mineral water group plasma tHcy reduced from 9.06 ± 2.08 µmol/L to 8.47 ± 1.42 µmol/L, while the beer group increased from 11.67 ± 2.17 to 14.58 ± 1.58 µmol/L, red wine group increased from 12.69 ± 1.85 to 15.61 ± 1.83 µmol/L and the spirit group increased from 13.8 ± 2.22 to 16.25 ± 2.19 µmol/L.

Stress

de Oliveira *et al.* [18] demonstrated with animal studies, which can be extrapolated to human pathology, that plasma tHcy concentrations were significantly altered by stress as compared to the control group by +38% (P=0.006) [i.e. mean 5.3µmol/L to mean 7.31µmol/L].

Smoking

Research shows that subjects who smoked 35.9 ± 6.4 cigarettes daily and who ceased smoking, homocysteine levels decreased from 8.58 ± 2.31 to 7.53 ± 2.26 µmol/L (P = 0.013). Significant changes in homocysteine levels were not observed in subjects who reduced smoking or continued to smoke [19].

HHCY and CVD

In essence, CVD refers to those diseases related to atheroma, arteriosclerosis and atherosclerosis. All three terms refer to pathophysiological changes in the

blood vessels. McCully was first to propose that HHCY may be a vascular factor. McCully's hypothesis was confirmed by subsequent studies by Refsum *et al.* [20] and Welch & Loscalzo [21]. linking HHCY to PAD, stroke, and coronary artery disease.

There is now an apparent abundance of evidence which demonstrates that there is a link between CVD and elevated homocysteine. However, Khandanpour *et al.* [22] only found weak evidence on the clinical beneficial effects of folate supplementation in PAD. Furthermore, Ford *et al.* [23] supports a role for homocysteine in the pathogenesis of CHD, but concluded that more epidemiological studies are needed.

Humphrey *et al.* [24] and Ueland *et al.* [25] have demonstrated that each increase of 5 µmol/L in homocysteine increases the risk of CHD by approximately 20%, independently of traditional CHD risk factors. It is well documented [26-28] that the risk for CHD is represented by a continuum of homocysteine concentrations with a substantial risk occurring at ≥10 µmol/L [26-28]. Robinson *et al.* [29] reports that any homocysteine level over 6.3µmol/L represents an increase risk of CHD.

Impaired Renal Function

Studies indicate that the kidneys account for approximately 70% of plasma clearance of homocysteine [30]. A common cause of hyperhomocysteinemia is a decline in renal function [31]. Studies have reported a strong inverse relationship between glomerular filtration rate and plasma tHcy levels [31].

Evidence Supporting the Pathology of Heightened Homocysteine and CVD

Excess homocysteine promotes CVD due to the following possible mechanisms:

Homocysteine has a reactive product, HCYT. This compound converts LDL to form small dense particles associated with increased susceptibility to vascular disease. HCYT also causes platelets to aggregate. Furthermore, HCYT becomes linked to the apoB protein of LDL by peptide-bound homocysteinyl groups, causing aggregation and precipitation of the LDL particles. The homocysteine-LDL aggregates are taken up by macrophages to form foam cells. In the artery wall, foam cells lead to deposition of cholesterol and fats creating arteriosclerotic plaques within the arterial walls [20, 32-37].

HCYT contributes to changes in coagulation factor levels so as to encourage blood clot formation [20, 32-37].

HCYT is believed to prevent small arteries from dilating so they are more vulnerable to obstruction [20, 32-37].

HCYT causes smooth muscle cells in the arterial wall to proliferate [20, 32-37].

HCYT generates superoxide and hydrogen peroxide, which have been linked to damage to arterial endothelium and enhances thrombogenicity [21, 38].

It has been shown that raised homocysteine depletes magnesium, causing calcium to flood into smooth muscle cells of vascular vessels that result in vasospasms that raise blood pressure and can lead to angina and heart attack [38-41].

In general, epidemiologic studies show an independent and graded association between homocysteine levels and cardiovascular risk [42-44]. Moreover, Boushey *et al.* [43] has demonstrated that a 5µmol/L increase in plasma tHcy is associated with an increased risk of CVD by an odds ratio of 1.60 in men and 1.80 for women.

Further Considerations

Brattstrom and Wilcken [45] concluded that much of the epidemiologic association found in prospective and retrospective studies as well as cross-sectional studies between modestly elevated plasma tHcy and cardiovascular risk or severity of atherosclerosis is often explained by renal mechanism malfunction. This constitutes the basis of their hypothesis, which implies that moderate HHCY in association with atherosclerotic CVD is a consequence but not a cause of the disease. There is however, considerable epidemiologic evidence that moderate HHCY is a risk factor for venous thromboembolic disease, as reviewed by a meta-analysis conducted by Ray [46] supported by Eichinger *et al.* [47] and Langman *et al.* [48]. Moreover, a correlation between HHCY and arterial vascular disease is well established [49, 50].

MATERIALS AND METHODS

Evidence in Research Publications

A computer assisted literature search was made for relevant studies using the search term

Table 1: Details of Selected Systematic Reviews, Meta-Analyses and other Studies on Hyperhomocysteinemia and Cardiovascular Disease Covering Twelve Different Types of chronic Conditions

Title of review Author's name Reference	Number of Eligible Studies	Number of Participants involved	Conclusions	Type
Plasma tHcy, folate, and vitamin B12 concentrations and risk for early-onset coronary artery disease. Pancharuniti *et al.* [56].	1	209	Elevated tHcy are associated with increase risk of CAD. It is unclear whether there is a threshold below which tHcy concentration does not pose a risk. Reduction in plasma tHcy may reduce CAD risk.	Case control study
A quantitative assessment of plasma homocysteine as a risk factor for vascular disease Boushey *et al* [43].	28	6433	A 5µmol/L increase in plasma tHcy is associated with an increase risk of CAD by an odds ratio of 1.6(95% CI, 1.4 to 1.7) for men and 1.8(95% CI, 1.4 to 2.3) for women. It is also associated with an increase risk of cerebrovascular disease by an odds ratio of 1.5(95% CI, 1.3 to 1.9). Hyperhomocysteinemia appears to act independently of other risk factors and is therefore considered an independent graded risk factor for arteriosclerotic vascular disease.	5 Cross sectional 23 Case controlled
Hyperhomocysteinemia and low pyridoxal phosphate common and independent reversible risk factors for CVD. Robinson *et al.* [29].	1	535	The risk of CHD rises with increasing plasma homocysteine regardless of age and sex, with no threshold effect.	Cohort case study
Homocysteine metabolism and risk of myocardial infarction: relation with vitamin B6, B12 and folate. myocardial Verhoef *et al.* [59].	1	248	Adds further epidemiologic evidence to the hypothesis that plasma homocysteine is an important independent risk factor for coronary disease. There is strong direct linear trend of plasma tHcy with the risk of infarction.	Case control study
Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. Ray [46].	9	487	A significant risk for venous thromboembolic disease in the presence of hyperhomocysteinemia apparently exists among a spectrum of patients with first or recurrent venous thromboembolic events. The risk appears to be most significant for patients < 60 years old.	Meta-analysis
Homocysteine and cardiovascular disease: a critical review of the epidemiologic evidence. Eikelboom *et al.* [44].	9	2690	A substantial body of epidemiologic evidence suggests an association between cardiovascular risk and moderately increased plasma tHcy levels.	Systematic review.
The controversy over homocysteine and cardiovascular risk. Ueland *et al*. [25].	14	13980	Hyperhomocysteinemia and preclinical atherosclerosis suggest that elevated plasma total homocysteine is a causal risk factor for cardiovascular disease including venous thrombosis.	Meta-analysis
Hyperhomocysteinemia and increased risk of venous thromboembolism. Langman *et al.* [48].	1	280	Hyperhomocysteinemia is a significant risk factor for venous thromboembolic disease. It is likely that hyperhomocysteinemia plays a causative role in the development of venous thrombosis.	Case-control study
Total plasma homocysteine level and risk of cardiovascular disease A meta-analysis of prospective cohort studies. Bautista *et al.* [52].	14	9834	Hyperhomocysteinemia moderately increases the risk of a first cardiovascular event, regardless of age and follow-up duration.	Meta-analysis

(Table 1). Continued.

Title of review Author's name Reference	Number of Eligible Studies	Number of Participants involved	Conclusions	Type
Homocysteine and cardiovascular disease: a systematic review of the evidence on case-control studies and nested case-control studies. Ford *et al* [23].	57	12855	Homocysteine concentration is only weakly related to coronary heart disease and somewhat more strongly related to cerebrovascular disease. Although other lines of evidence support a role for homocysteine in the pathogenesis of cardiovascular disease, more information from prospective epidemiological studies or clinical trials is needed to clarify this role.	Systematic review
Homocysteine and risk of ischaemic heart disease and stroke: a meta-analysis The Homocysteine Studies Collaboration. [54].	30	6186	Lowering plasma homocysteine by 3 µmol/L was accompanied by a lower risk of ischaemic heart disease by11% and by a lower risk of stroke by 19% The meta-analysis suggests that elevated. homocysteine is at most a modest independent predictor of ischaemic heart disease and risk of stroke in a healthy population.	Meta-analysis
Homocysteine and cardiovascular disease: evidence on causality from by a meta-analysis Wald *et al.* [42].	92	20669	Lowering homocysteine concentrations by 3 µmol/L would reduce the risk of ischaemic heart disease by 16%, deep vein thrombosis 25% and stroke by 24%.	Meta-analysis
Prospective study of serum homocysteine and risk of ischemic stroke among patients with pre-existing coronary heart disease. Tanne *et al.* [53].	Not stated	160	Serum total homocysteine concentration is a strong predictor for incident ischemic stroke among patients at increased risk because of chronic coronary heart disease. The graded association observed is independent of traditional risk factors or markers inflammatory and indicates the importance of serum homocysteine levels in patients with pre-existing vascular disease.	Case control study
Hyperhomocysteinemia as a risk factor for coronary atherosclerotic disease in the elderly. Gravina-Taddei *et al.* [68].	1	172	Hyperhomocysteinemia was an independent risk factor for coronary artery disease in elderly individuals.	Case control study
Homocysteine lowering with B vitamins in vascular disease. The Heart Outcome Prevention Evaluation (HOPE2) Investigation, [62].	1	5522	Supplements combining vitamin B12 and folic acid did reduce homocysteine, but apparently did not reduce the risk of major cardiovascular events in patients with vascular disease.	Cohort case study
Folic acid improves vascular reactivity in humans: a meta-meta- analysis of RCT's. de Bree *et al.* [60].	14	732	Folic acid administration beneficially affects endothelial function by lowering homocysteine concentration, which potentially reduces risk of cardiovascular. disease.	Meta-analysis
Efficacy of folic acid supplementation in stroke prevention: A meta-analysis. Wang *et al.* [58].	8	16841	The meta-analysis provides coherent evidence that folic acid supplementation can significantly reduce risk of stroke in primary prevention by its effect on plasma homocysteine concentrations.	Meta-analysis
Risk prediction, homocysteine in coronary heart disease. Govindaraju & Manjunath [57].	1	273	Elevated homocysteine is related to CAD and is the best predictor with an odds ratio of 83.2 amongst other conventional risk factor in CAD patients.	Case control study
Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis Humphrey *et al.* [24].	26	Not stated	Each increase of 5 µmol/L in homocysteine level increases the risk of coronary heart disease events by 20%, independently of traditional coronary heart disease risk factors.	Systematic review and meta-analysis

(Table 1). Continued.

Title of review Author's name Reference	Number of Eligible Studies	Number of Participants involved	Conclusions	Type
Homocysteine and peripheral arterial stated disease: systematic review and meta-analysis Khandanpour *et al*. [22].	33	Not	Patients with peripheral arterial disease have significantly higher homocysteine levels than unaffected controls. However, no robust evidence on clinically beneficial effects of folate supplementation in peripheral arterial disease was found.	Systematic review and meta-analysis
Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality. Clarke *et al* [63].	8	37485	Lowering homocysteine levels by 25% has no significant effect on the incident of major vascular events, over a five year period.	Meta-analysis
Homocysteine and reclassification of cardiovascular disease risk. Veeranna *et al*. [55].	2	13247	From these two disparate population cohorts, it was found that the addition of Hcy level to Framingham Risk Score significantly improved risk prediction, especially in individuals at intermediate risk of CHD. events.	Meta-analysis
Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication bias Clarke *et al.* [67].	19	116136	The overall result from large unpublished datasets shows lifelong moderate homocysteine elevation has little or no effect on CHD. The discrepant overall result from previously published studies reflects publication bias or methodological problems.	Meta-analysis
C1 metabolism and cardiovascular disease in older adults McNulty *et al.* [61].	6	29653	Most of the RCTs designed to show a causative relationship between outcomes total homocysteine and cardiovascular disease provide little evidence that giving B-vitamins to cardiovascular disease patients prevent another event. The same cannot be said for prevention. Emerging nutrition policy should consider nutrient requirements aimed at primary prevention as it remains probable that there is a role for B - vitamins in the primary prevention of stroke.	RCT

"Hyperhomocysteinemia", "elevated homocysteine and cardiovascular disease", which revealed 379 entries related to the period January 1994 to May 2013. From these entries just twenty four (282,727 participants) met the initial set criteria.

This was followed by a methodological quality assessment conducted by two authors employing AMSTAR, which is a one-page tool comprising eleven questions that checks the quality of the studies and the level of agreement obtained by the reviewers. Each question was scored as one point for a 'Yes' answer and a score of zero for all other answers. The outcome was statistically analysed employing Kappa (k) statistics. A calculation was made to establish the mean k at 95% (CI) employing Student's t test. The non-systematic reviews/meta-analyses were assessed by one author and checked by another for methodological validity employing standardised data extraction tools from JB1000308 [51].

RESULTS

The search revealed eleven meta-analyses, one systematic review, two systematic review and meta-analyses, and ten other studies, see Table **1**.

The fourteen initially screened systematic reviews/meta-analyses studies mean k, 95% (CI) was 0.77, which scored a Kappa interpretation of 'Substantial Agreement', between the reviewers, presented in Table **2**. The remaining studies, met the requirements of the extraction tools from JB1000308 [51].

Twenty four CVD conditions (82.8%) of the twenty four studies examined, demonstrate that epidemiologic and clinical data indicate that elevated plasma tHcy is a risk factor for CVD [22-25, 29, 42-44, 46, 48, 52-58].

Twenty CVD conditions (71.4%) of the twenty four studies found that plasma tHcy levels can be employed

Table 2: Kappa (k) Statistic for Agreement for each AMSTAR Question and Proportion of "Yes" Answers, Together with Interpretation of Kappa

AMSTAR question? studies)	k (95% CI)	Reviews scoring an agreed "yes" (out of 15 (%)	Interpretation of kappa [69,70]
1. A priori design?	1.0 (1.0,1.0)	40	Almost perfect agreement
2. Duplicate study selection and data abstraction?	0.51 (0.1,0.92)	33	Fair agreement
3. Comprehensive literature search?	0.55 (0.1,1.0)	60	Moderate agreement
4. Publication status used for inclusion?	0.59 (0.18,1.0)	53	Moderate agreement
5. Listed included and excluded studies?	1.0 (1.0,1.0)	10	Almost perfect agreement
6. Characteristics of included studies tabulated?	1.0 (1.0,1.0)	93	Almost perfect agreement
7. Scientific quality of included studies assessed?	0.61 (0.22,1.0)	33	Substantial agreement
8. Scientific quality appropriately to formulated conclusions?	0.61 (0.22,1.0)	33	Substantial agreement
9. Appropriate methods used to combine studies?	1.0 (1.0,1.0)	80	Almost perfect agreement
10. Likelihood of publication bias assessed?	0.55 (0.1,1.0)	20	Moderate agreement
11. Conflict of interest included?	1.0 (1.0,1.0)	50	Almost perfect agreement
Mean (k) 95% (CI)	0.77 (0.62,0.92)		Substantial agreement

Table **2** outlines the results of the agreement reached by the reviewers of the fourteen systematic reviews/meta-analyses studies that met the initial set criteria, with each study being addressed by the eleven AMSTAR questions.

as a biomarker for the risk of CVD [22, 24, 25, 29, 42, 43, 46, 48, 52-59].

Ueland *et al.* [25]; Ubbink [27]; Stanger *et al.* [28]; Robinson *et al.* [29] and Boushey *et al.* [43] demonstrated that the odds ratio for the risk of developing CHD increases with increasing plasma tHcy. The results are presented in Figure **2**.

Twelve CVD conditions (46.2%) of the twenty four studies indicated that reducing elevated plasma tHcy reduces the risk of CVD [24, 42, 43, 53 54, 56, 58, 60, 61], whilst 23.1% of the studies did not support this [22, 44, 53, 62, 67]. The remaining studies reached no conclusion or did not comment [23, 25, 29, 46, 48, 52, 59, 68].

DISCUSSION

Cardiovascular Disease

Bazzano *et al.* [65] found that there was no evidence to support that reducing homocysteine reduced the risk of CVD. However, a subsequent study conducted by Bleie [66] demonstrated that two years of homocysteine-lowering treatment with moderate doses of folic acid and vitamin B12 improved coronary blood flow, reflecting improved coronary vascular function. Ray [46] demonstrated a significant risk for venous thromboembolic disease in the presence of HHCY, with the risk most significant for patients before the age of 60 years. Boushey *et al.* [43] demonstrated that a 5μmol/L increase in plasma tHcy is associated with an increased risk of CVD by an odds ratio of 1.6 in men and 1.8 for women; furthermore, it increased the risk associated with cerebrovascular disease by an odds ratio of 1.5 in both gender. Elevated plasma tHcy was also shown to be an independent graded risk factor for arteriosclerotic vascular disease. McNulty *et al.* [61] concluded that emerging nutrition policy should consider nutrient requirements aimed at prevention of primary CVD events, as it remained probable that there is a role for B-vitamins in the prevention of primary stroke. Veeranna *et al.* [55] demonstrated that the results of their study lend support to previously published data exploring the association between Hcy and CVD and CHD events. In contrast to other studies Clarke *et al.* [67] found that from unpublished studies that lifelong moderate homocysteine elevation had little or no effect on CHD of MTHFR case control studies. It is assumed here that moderate homocysteine elevation

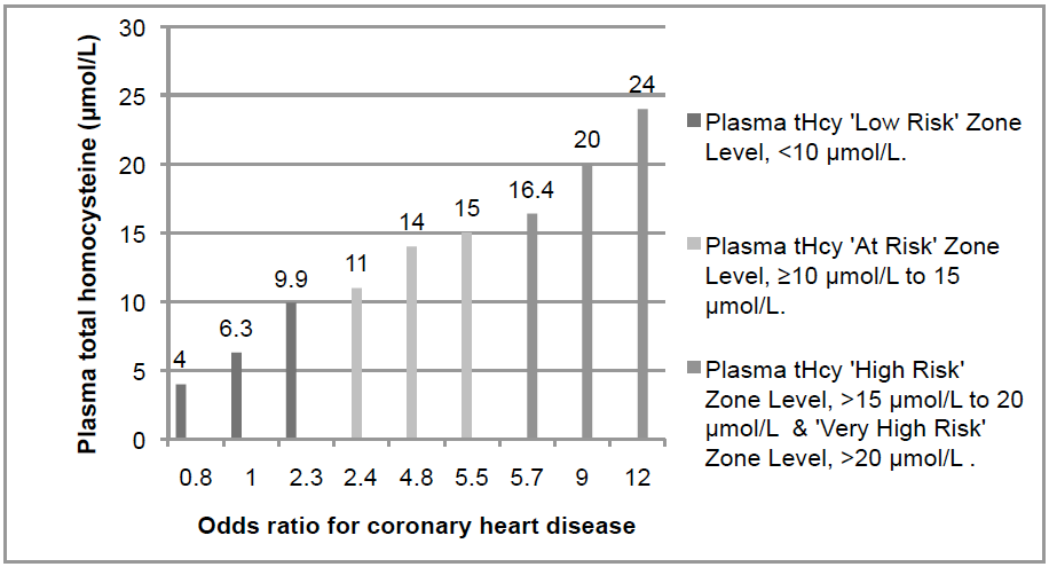

Figure 2: The odds ratio for coronary heart disease compared to plasma total homocysteine.

is equivalent to 15 to 30 µmol/L [3, 20]. However, no reference was made to homocysteine elevation being higher than this. In addition, Clarke and colleagues findings reveal a claimed serious example of publication bias and argue against the use of folate supplements as a means of reducing CHD risk.

Factors Contributing to Increased Plasma tHcy

Unhealthy lifestyle, a low intake of B-vitamins, gastrointestinal malabsorption of vitamins, and drug interactions are the most frequent causes that lead to HHCY. Notably, age and gender have a bearing on homocysteine levels. Obersby *et al.* [9] demonstrated that vegetarians usually have elevated plasma tHcy levels and are therefore susceptible to developing elevated homocysteine related CVD. As an consequence, diet can significantly affect levels also. Smoking is strongly associated with increased homocysteine levels [64]. High consumption of coffee, inadequate nutrition, lack of physical exercise, and stress together with alcohol are all associated with elevated homocysteine levels.

Severe hyperhomocysteinemia can be caused by impaired renal function and a rare autosomal recessive genetic disorder due to a deficiency in the enzyme CBS causing homocysteine to rise sharply. It can also be caused more commonly by mutations of the enzyme MTHFR allowing homocysteine to rise. The findings show that diet, supplementation and lifestyle can have a profound effect on levels of plasma tHcy. A diet rich in homocysteine-lowering nutrients containing vegetables, fruits, legumes, lean meat, fish, whole grains and cereals together with maintaining a healthy lifestyle is a good foundation for healthy levels of homocysteine. It is unlikely that modifications to an unhealthy diet and lifestyle alone would normalise levels of intermediate and severe HHCY classifications without the use of supplements. The findings of the present study show that there is an inverse relationship between folic acid, vitamin B12 and plasma tHcy. It is widely accepted that, at a cellular level, homocysteine exerts a detrimental effect on arterial walls. In addition its reactive product, HCYT, encourages clot formation and generates superoxide and hydrogen peroxide which enhances thrombogenicity. Epidemiological studies have clearly indicated that plasma tHcy is an independent risk factor for atherosclerosis. Raised homocysteine depletes magnesium that can elevate blood pressure through vasoconstriction. There is considerable epidemiologic evidence and clinical data available to support the hypothesis that levels of plasma tHcy can be employed as a major independent risk factor for CVD.

Taken together, the data indicates that elevated plasma tHcy is contributory to many aspects of CVD, and can be employed as an independent biomarker. Furthermore, the difficult to prove hypothesis that reducing plasma tHcy lowers the risk of some CVD events remains unclear, although 46.2% of the CVD conditions in the studies found that reducing plasma tHcy reduces the risk of a considerable amount of CVD events, and it is therefore prudent to take steps to aim for normal levels of plasma tHcy, as a precaution to avoid the risk of developing or exacerbating CVD.

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ACRONYMS

AMSTAR = Assessment of Multiple Systematic Reviews

CBS = Cystathionine β-synthase

CHD = Coronary heart disease

CVD = Cardiovascular disease

FDA = Food and drug administration

HCYT = Homocysteine thiolactone

HHCY = Hyperhomocysteinemia

JBI = Joanna Briggs Institute

LDL = Low-density lipoproteins

LV-LOV's = Lactovegetarians or lactoovovegetarians

MTHFR = Methylenetetrahydrofolate reductase

PAD = Peripheral arterial disease

SAM = S-adenosylmethionine

tHcy = Total homocysteine

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