Published in final edited form as:

Cardiovasc Toxicol. 2009 June ; 9(2): 53–63. doi:10.1007/s12012-009-9042-6.

Quo Vadis: Whither Homocysteine Research?

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Abstract

Four decades of research on the link between hyperhomocysteinemia and cardiovascular disease has led to a crossroads. Several negative studies on the role of homocysteine-lowering B-vitamin therapy in reducing the risk of atherothrombotic cardiovascular disease have dampened enthusiasm for this important field of research. In this review, we assess the present state of homocysteine research and suggest potential avenues that would help to clarify the purported link between the plasma homocysteine level and cardiovascular risk. We address several questions raised by the findings of various basic, epidemiological and clinical studies and attempt to construct a framework that we believe will allow us to address the fundamental unresolved issues in this controversial area, specifically focusing on the risk of coronary vascular disease and cardiac failure. This review should allow researchers to deconstruct this complex field into separate areas that, when addressed adequately, may lead to findings that elucidate the overall link between hyperhomocysteinemia and cardiovascular disease and allow the design of appropriate clinical trials.

Keywords

Homocysteine; Cardiovascular disease; Heart failure; Oxidant stress; Cardiac remodeling

Introduction

“If we could first know where we are, and whither we are tending, we could better judge what to do, and how to do it.”

—Abraham Lincoln, “House Divided” Speech, Springfield, Illinois, June 16, 1858.

Since the original proposition by Kilmer McCully [1] that an elevated plasma homocysteine level is a potential risk factor for atherothrombotic cardiovascular disease, numerous experimental, clinical, and epidemiological studies have addressed this interesting link. Four decades later, this hypothesis has not been conclusively confirmed or refuted. Recent

clinical trials that examined whether combined B-vitamin therapy will decrease cardiovascular events in subjects with established cardiovascular disease have yielded “disappointing” results. In this review, we will address the contradictory results of the past four decades and propose that further research in a systematic manner would be needed before fulfilling or negating Koch’s postulates in this field. The first part of this article will present a focused review of relevant data on the link between hyperhomocysteinemia and cardiovascular disease, and the second section will discuss questions raised by the current data and propose avenues of research.

Hyperhomocysteinemia and Cardiovascular Disease

Plasma Homocysteine Level and Cardiovascular Disease: Promising Observational Data Versus Negative Clinical Trials

Meta-analyses—McCully proposed the link between homocysteine and cardiovascular disease based on observations made on autopsy studies of young subjects with homocystinuria who had died from atherothrombotic complications [1]. Since these seminal observations, investigators worldwide have conducted numerous observational studies to explore the association between mild to moderate elevations of plasma homocysteine level and the risk of cardiovascular disease. Boushey and coworkers conducted a meta-analysis of 27 studies that included prospective and cross-sectional observations [2]. The meta-analysis also included studies that examined the effect of folic acid. The authors reported a consistent association of plasma homocysteine to cardiovascular disease across studies conducted in different populations analyzed with varying methodologies. An increase in plasma homocysteine level of 5 $\mu\text{mol/l}$ was associated with an odds ratio (OR) for coronary artery disease of 1.6 for men and 1.8 for women, and an OR of 1.5 for cerebrovascular disease; ~10% of the risk of coronary artery disease in the population was attributed to the homocysteine level by this analysis. The study also demonstrated that folate supplementation could reduce homocysteine levels; interestingly, the impact was greater for food fortification with folate compared to folate supplementation itself. Another meta-analysis conducted by the Homocysteine Studies Collaboration group included 30 studies reported between 1966 and 1999 [3]. Stronger associations were seen in retrospective compared to prospective studies. The authors concluded that a 25% lower plasma homocysteine level was associated with 11% reduction in the risk of coronary artery disease and 19% reduction in stroke risk.

A major influence on the plasma homocysteine levels in the general population is the activity of methylene tetrahydrofolate reductase, a key enzyme in folic acid metabolism. A common polymorphism in the gene coding for this enzyme results from a cytosine $\rightarrow$ thymidine substitution. This polymorphism is highly prevalent in the general population, with 10% being homozygous (TT), 43% heterozygous (CT), and 47% unaffected (CC). Wald and coworkers conducted a meta-analysis of 72 studies in which this mutation was analyzed and of 20 prospective studies that examined the association between homocysteine level and cardiovascular risk without genetic analysis [4]. The rationale of this meta-analysis was to determine whether associations between the homocysteine level and cardiovascular risk were similar when analyzing an increase in homocysteine level due to a specific genetic mutation (in which the cardiovascular risk factors are expected to be similar between different genetic groups) or in the presence of multiple causes and confounding factors as in the general population. Interestingly, in the genetic studies, the OR for ischemic heart disease in the TT genotype compared to CC genotype was 1.21 ($P = 0.0006$), while between the heterozygous CT genotype and unaffected CC genotype was 1.06. There was a corresponding increase in the plasma homocysteine level: the level in the TT group was 2.7 $\mu\text{mol/l}$ higher than in the CC group, while the level in the CT group was 0.29 $\mu\text{mol/l}$ higher than in the CC group. In the prospective studies, after adjusting for multiple confounding

variables, the OR was found to be 1.32 for a 5 $\mu\text{mol/l}$ increase in the plasma homocysteine concentration. The similarity between these two groups of meta-analysis as to the attributable risk of plasma homocysteine level suggests a strong association that occurs despite different sets of confounding variables, and suggests, but does not prove, a possible causal association of elevated plasma homocysteine level with cardiovascular disease.

Prospective Clinical Trials—The observational studies mentioned earlier raised the obvious need for large, randomized controlled studies to examine whether homocysteine-lowering therapy would decrease the risk of cardiovascular disease. Table 1 describes relevant details from the major studies conducted over the last decade in chronological order. The Vitamin Intervention for Stroke Prevention (VISP) trial [5] randomized 3,680 subjects with nondisabling cerebral infarction to low or high doses of the combination of vitamins B₆, B₁₂, and folate (there was no placebo group). A small reduction of plasma homocysteine level by 2 $\mu\text{mol/l}$ was achieved in the high dose vitamin group compared to low dose group, but there was no effect on recurrent cerebral infarction (primary outcome) or on coronary events and death (secondary outcome). An interesting subanalysis examined the relation between the baseline homocysteine level and outcomes. In the low-dose treatment group, a 3 $\mu\text{mol/l}$ lower baseline level was associated with 10% lower risk of stroke ($P = 0.05$), a 26% decrease in risk of coronary events ($P < 0.001$), and a 16% lower risk of death ($P = 0.001$). The high-dose group, interestingly, showed a nonsignificant lower risk of 2% for stroke, 7% for coronary events, and 7% for mortality.

The NORVIT trial was a secondary prevention trial that enrolled 3,749 subjects who had a recent myocardial infarction [6]. Subjects were randomized to one of four groups: folic acid, vitamin B₆, and vitamin B₁₂; folic acid and vitamin B₁₂; vitamin B₆; or placebo. The composite primary end-point of recurrent myocardial infarction, stroke, and sudden death due to coronary disease was not decreased by treatment; in fact, there was a trend towards a worse outcome in the group given the combination of folic acid, vitamin B₆, and vitamin B₁₂. The HOPE 2 trial [7] also targeted patients at risk of vascular events (documented vascular disease or diabetes mellitus). In this study, 5,522 subjects were randomized to placebo or the combination of folic acid, vitamin B₆, and vitamin B₁₂. Mean plasma homocysteine levels were lowered by a mean value of 2.4 $\mu\text{mol/l}$ in the active treatment group as opposed to an increase of 0.8 $\mu\text{mol/l}$ in the placebo group. Over a 5-year follow-up period, homocysteine-lowering therapy did not significantly reduce the composite primary end point of death from cardiovascular causes, myocardial infarction, and stroke. Interestingly, stroke rates were reduced in the active treatment group, while hospitalization for angina was increased in the treatment group.

Patients with renal dysfunction have a high incidence of cardiovascular disease as well as higher levels of plasma homocysteine. The HOST study examined the hypothesis that lowering homocysteine level in patients with chronic kidney disease would lower the risk of cardiovascular events [8]. In this randomized study conducted on 2,056 male subjects, treatment with high doses of folic acid, vitamin B₆, and vitamin B₁₂ lowered plasma homocysteine level by 6.3 $\mu\text{mol/l}$ (25.8% reduction), but did not lower total mortality or cardiovascular events. Another study that focused on a specific patient population was the WAFACS study, which examined the effect of homocysteine lowering therapy in women [9]. In this study, 5,442 women aged 42 years or older, who were US health professionals and had either documented cardiovascular disease or had three or more coronary risk factors, were randomized to placebo or the combination of folic acid, vitamin B₆, and vitamin B₁₂. As with the previously described studies, a lowering of plasma homocysteine was not associated with reduction in cardiovascular events over 7.3 years of follow-up. A recently published study from Norway in 3,096 subjects undergoing coronary angiography examined the effect of placebo, vitamin B₆ alone, folic acid and vitamin B₁₂ administered

together, or the combination of folic acid and vitamins B₆ and B₁₂ [10]. As with the other studies, there was no effect of any vitamin therapy on total mortality or cardiovascular events.

The contrast between the strong associations seen on observational and prospective studies and the lack of effect of homocysteine-lowering therapy on cardiovascular events in prospective, randomized, and controlled studies raises the question whether homocysteine is a marker for disease and not a causative factor, as has been discussed in a recent review [11]. Alternatively, are we being too simplistic in trying to fulfill Koch's postulates in the setting of a very complex metabolic derangement that does not lend itself to easy therapeutic solutions? Answering this with a Socratic approach will be the object of this review.

Hyperhomocysteinemia is a Complex Metabolic Derangement with Multiple Pathogenic Mechanisms

Figure 1 shows a synoptic view of the “methionine–homocysteine axis”, which is connected to various biologic modules that could contribute to pathogenesis. The cycle starts with methionine, an essential amino-acid that reacts with adenosine triphosphate in a reaction catalyzed by methionine adenosyl transferase to form the major methyl donor *S*-adenosyl methionine. Methyl transferases catalyze the crucial reaction in which *S*-adenosyl methionine donates a methyl group to various molecules, including DNA, RNA, and proteins, and, in the process, is demethylated to *S*-adenosyl homocysteine. *S*-adenosyl homocysteine is the precursor of all the homocysteine produced in the body, which is generated by a hydrolytic reaction catalyzed by the enzyme *S*-adenosyl homocysteine hydrolase. Homocysteine, thus produced, can be either remethylated to methionine or transsulfurated to cysteine or sulfates. Remethylation occurs through two enzymatic pathways: one catalyzed by methionine synthase, which utilizes 5-methyl tetrahydrofolate as the methyl donor and vitamin B₁₂ as a cofactor, or a reaction catalyzed by betaine-homocysteine methyl transferase (active only in the liver) in which betaine acts as the methyl donor. The transsulfuration pathway is initiated by the enzyme cystathionine beta synthase, which requires vitamin B₆ as a cofactor. This pathway, which has limited tissue distribution, can lead to the formation of cysteine and subsequently the major antioxidant molecule glutathione or can lead to the elimination of homocysteine from the body as taurine or inorganic sulfate. As Fig. 1 illustrates, the methionine–homocysteine axis is connected to methylation, one carbon metabolism via folate; and to cellular redox status, via glutathione, in the normal state.

Figure 1 clearly illustrates that an elevated plasma homocysteine level is the tip of a metabolic iceberg, beneath which is a complex set of interrelated metabolic consequences. We refer the reader to reviews that deal extensively with this subject [11, 12]. An elevation of plasma homocysteine could result from various factors, such as an excess of methionine in the diet (a popular animal model); deficiencies of vitamins such as folate, B₁₂ and B₆; or inherited deficiencies of enzymes involved in the methionine–homocysteine cycle, such as cystathionine beta synthase, or of enzymes involved in folate metabolism, such as methylene tetrahydrofolate reductase. Not only can an elevated plasma homocysteine level suggests multiple mechanisms of origin, but it also suggests multiple pathogenic mechanisms that could be operative, as shown in Fig. 1. A high homocysteine level could lead to an elevation of *S*-adenosyl homocysteine compared to *S*-adenosyl methionine, a situation that would decrease methylation reactions and lead to a state of “hypomethylation”.

Hyperhomocysteinemia can lead to increased oxidant stress by a number of mechanisms [13]. Homocysteine can undergo oxidation to the disulfide form homocystine and, in the process, generates reactive oxygen species. We have shown that homocysteine decreases the activity of the major cellular antioxidant enzyme glutathione peroxidase (GPx)-1 by interfering with translation [14]. Homocysteine can also increase the activity of the

prooxidant enzyme NADPH oxidase [15]. The prooxidant environment created by hyperhomocysteinemia can also decrease the bioavailability of nitric oxide, with deleterious effects on vascular function. Yet, homocysteine can lead to generation of glutathione through the transsulfuration pathway (Fig. 1). Glutathione is a major antioxidant molecule, a substrate for glutathione transferases and peroxidases, and it plays a major role in regulating cellular processes, including proliferation, differentiation, and apoptosis. Hence, changes in homocysteine levels could influence cell phenotype and the pathogenesis of cardiovascular disease, in part, by altering cellular glutathione levels.

Another mechanism of homocysteine toxicity involves the direct valent incorporation of homocysteine into proteins, or homocysteinylation, which can alter protein function. This posttranslational modification can occur by the reaction of thiol group of homocysteine with thiol groups of cysteine residues in proteins, as shown by Jacobsen and colleagues [16]. This can also occur by N-homocysteinylation, described by Jakubowski and colleagues [17]. For N-homocysteinylation to occur, homocysteine has to be converted to homocysteine thiolactone through a reaction involving methionyl tRNA synthetase. Homocysteine thiolactone reacts with the amino group of lysine residues in proteins to create N-homocysteinylated proteins. Interestingly, since methionyl tRNA synthetase is involved in the process, there is an inverse relation between methionine levels and N-homocysteinylation.

Thus, it is amply clear that we cannot conceive of hyperhomocysteinemia as a pathologic state with a single mechanism of adverse action. An elevated plasma homocysteine level could signify either altered methylation status, altered redox status, altered protein status due to homocysteinylation, or combinations thereof. Hence, our approach to reverse or prevent pathology in hyperhomocysteinemic States should also take into account the potential diversity of pathobiological mechanisms in subjects with a similar range of plasma homocysteine levels.

Novel Cardiovascular Targets for Hyperhomocysteinemia: Myocardial Structure and Function

Based on the hypothesis initially proposed by McCully, most experimental and clinical works on hyperhomocysteinemia and cardiovascular disease have focused on vascular disease. However, as detailed in a review cited earlier [12], there is reason to believe that the biological mechanisms postulated for vascular pathology of hyperhomocysteinemia could promote adverse myocardial remodeling and failure. Specifically, the effects of homocysteine on vascular smooth muscle cell proliferation and collagen production could lead to reactive myocardial fibrosis and cardiac dysfunction, while oxidant stress is a major mediator of cardiomyocyte injury and survival. Experimental studies from our laboratory and those of others show that an elevated plasma homocysteine level is associated with adverse cardiac structure and function [18-22]. Reactive myocardial fibrosis, with coronary arteriolar remodeling and perivascular and interstitial fibrosis, is a major component of hyperhomocysteinemia-induced myocardial changes. Hyperhomocysteinemia-induced myocardial changes not only provoke abnormal myocardial function, *de novo*, but also accelerate the progression of other potent pathological stimuli, such as hypertension. In terms of mechanisms, increased oxidant stress is associated with hyperhomocysteinemia-induced myocardial fibrosis. A recent study from our laboratory also demonstrates that hyperhomocysteinemia, by altering antioxidant defense mechanisms, can lead to increased cardiomyocyte death and cardiac dysfunction after ischemia-reperfusion injury (unpublished observation).

Investigators from the Framingham Heart Study have found that plasma homocysteine levels predicted the risk of overt heart failure, both systolic and diastolic, over a follow-up period

of approximately 8 years [23]. A recent report from the Framingham Heart Study showed that plasma homocysteine level was independently related to plasma markers of collagen metabolism in subjects with and without echocardiographic evidence of left ventricular remodeling [24]. Clinical studies have also shown an association of plasma homocysteine level with systolic dysfunction [25, 26]. Hence, a direct effect of hyperhomocysteinemia-induced pathogenic mechanisms on the myocardium could also account for the association of hyperhomocysteinemia with cardiovascular disease, in addition to atherothrombotic vascular events.

Whither Homocysteine Research?

Clearly, research in the field of homocysteine and cardiovascular disease is at a crossroads. Skepticism abounds as to whether plasma homocysteine is just a marker of underlying disease in view of the results of trials of homocysteine-lowering utilizing B-vitamins. We will attempt to frame the issues raised by four decades of research and propose avenues that may allow cardiovascular researchers a way forward.

A Very High Plasma Homocysteine Level Could Lead to Cardiovascular Disease; a Modestly Elevated Level Does Not

Severe hyperhomocysteinemia (over 100 $\mu\text{mol/l}$) secondary to inborn errors of metabolism such as a severe deficiency of cystathionine beta synthase (CBS), is associated with high rates of mortality from atherothrombotic vascular events at a young age. Treatment of such individuals with vitamin therapy lowers plasma homocysteine levels, and substantially lowers the rate of vascular events. Yap and coworkers reported the results of a multicenter, multinational, observational study of patients with homocystinuria treated with B vitamin therapy [27]. As it would be unethical to conduct a randomized placebo controlled study due to the reported effectiveness of homocysteine-lowering therapy in reducing the high vascular event rate in this population, the authors compared outcomes in treated patients to predicted outcomes based on the previous reports on the natural history of homocystinuria. Over 2,822 patient years of treatment, vascular events were reduced from an expected number of 112–17 (risk reduction of 91%). Plasma homocysteine levels were significantly reduced from pretreatment levels (more than 150 $\mu\text{mol/l}$), but remained moderately elevated on treatment.

What could be the explanation for this discrepancy between a marked reduction of vascular events despite reduced, but still moderately elevated plasma homocysteine levels? Could the 17 vascular events in 12 out of a total of 158 patients included in the study, despite a substantial reduction of plasma homocysteine levels, albeit not to normal levels, suggest that the association of vascular events with mildly elevated plasma homocysteine levels in the general population is due to a common undefined factor and not due to a direct vascular pathogenic effect of homocysteine? Alternatively, could we conclude that this study shows that high-dose vitamin therapy reduces vascular event rate in individuals with markedly elevated plasma homocysteine levels, but not in individuals with mildly elevated homocysteine levels, thereby necessitating a different approach in mild hyperhomocysteinemia (15–50 $\mu\text{mol/l}$)? We would argue that the second conclusion is a more valid interpretation of these data.

Primary Prevention Versus Secondary Prevention

Folate fortification was started in the United States in 1996 and fully implemented by 1998, while a similar policy was implemented in Canada in 1998. Yang and colleagues compared stroke mortality rates in the United States and Canada to the rates in England and Wales, where folate fortification is not mandatory [28]. As expected, folate levels increased and homocysteine concentrations decreased in the US after folate fortification. In this study,

stroke mortality rates were compared between two time periods: before folate fortification (1990–1997) and after folate fortification (1998–2002). The decline in stroke mortality in the US increased from -0.3% /year before fortification to -2.9% /year after fortification, while the rate of decline accelerated in Canada from -1.0% /year to -5.4% /year. There was no significant change in the decline in stroke mortality in England and Wales from 1990 to 2002. Sensitivity analysis to adjust for changes in other risk factors (using data from the National Health and Nutrition Examination Survey) indicated that changes in major, recognized risk factors did not account for the reduction in stroke-related deaths. Although this study does not prove cause and effect, it suggests that folate fortification was responsible, in some part, for the observed reduction in stroke mortality over time.

Bostom and colleagues predicted that the North American homocysteine-lowering trials (VISP, HOPE-2, WAF-ACS) would be handicapped by the introduction of folic acid fortification [29]. They suggested that the studies would achieve only 20–25% of the projected lowering of homocysteine levels (1.0 – 1.5 $\mu\text{mol/l}$ compared to 4.0 – 6.0 $\mu\text{mol/l}$). Hence, they concluded that the trials will be significantly underpowered to detect a significant treatment effect. They further tested this hypothesis in a cohort of 131 stable patients with coronary artery disease [30] and showed that homocysteine-lowering using folic acid-based vitamin treatment was modest (≈ 1.0 $\mu\text{mol/l}$). However, as Table 1 shows, the mean homocysteine lowering in these trials was greater, although it did not reach the 4.0 – 6.0 $\mu\text{mol/l}$ range. Furthermore, in the HOST trial (subjects with renal dysfunction), homocysteine lowering was substantial (≈ 6.0 $\mu\text{mol/l}$). In addition, the NORVIT and WENBIT trials were conducted in nonfortified populations. To deal with the fact that these trials may have been underpowered, the B-Vitamin Treatment Trialists' Collaboration suggested that a combined analysis of all the trials may have sufficient power to determine the effect of homocysteine-lowering therapy [31].

Overall, the results of the major trials published to date suggest the possibility that prolonged, albeit modest, homocysteine lowering with folate may prevent development or instability of atherosclerotic lesions, while secondary prevention utilizing 5–10 years of vitamin therapy in subjects with established vascular disease is not an effective strategy.

Could B-vitamin Therapy Have Competing Adverse Effects?

A small study by Lange and colleagues in subjects undergoing percutaneous coronary intervention showed that the combination of folate and vitamins B₆ and B₁₂ increased the rates of restenosis [32]. Similarly, in the NORVIT trial, outcomes were worse in the group given the same combination of B vitamins [6]. There are several mechanisms postulated for a potential adverse effect of B-vitamin therapy, which relate to the links among the methionine–homocysteine axis (Fig. 1), one-carbon metabolism, and methylation status [33]. Folate, by its effects on thymidine synthesis can promote cell proliferation and growth of the atherosclerotic plaque. Since methylation is dependent on the ratio of *S*-adenosyl methionine to *S*-adenosyl homocysteine, increasing methylation potential by decreasing homocysteine (and thereby *S*-adenosyl homocysteine levels) could increase generalized methylation. Since many genes with CpG islands in their promoter regions are influenced by methylation potential, this could potentially lead to changes in expression of proatherogenic molecules. In addition, the formation of asymmetric dimethyl arginine (ADMA), an inhibitor of nitric oxide synthase, is also increased by augmented methylation potential. As detailed in the review mentioned above by Antoniadou et al. [11], hyperhomocysteinemia is associated with increased synthesis of ADMA via activation of arginine-protein methyltransferases, as well as with decreased degradation of ADMA (to arginine) by downregulation of the enzyme dimethylarginine-dimethylaminohydrolase. Since ADMA is a powerful inhibitor of endothelial nitric oxide synthase, elevated ADMA levels can lead to decreased bioavailability of nitric oxide, and, thereby, to endothelial dysfunction. Hence, it

is possible that attempts to decrease homocysteine levels by B-vitamin therapy could have adverse effects that offset the benefit of lowering plasma homocysteine levels. This argues for the potential benefit of an approach that is based on identifying and targeting specific pathogenic mechanisms rather than imply the plasma homocysteine level.

Can Established Structural Changes be Reversed by Short-Term Therapy?

The negative results of large clinical trials raise another question: Can the accumulated effects of altered methionine–homocysteine metabolism on cardiac and vascular tissue over decades be reversed using relatively short periods of therapy? We addressed this issue in our dietary hyperhomocysteinemia-induced model in which 10 weeks of hyperhomocysteinemia led to myocardial fibrosis and worse diastolic function in spontaneously hypertensive rats [18]. In a recently published study, we examined whether extending exposure to altered methionine–homocysteine metabolism to 20 weeks would accelerate progression of hypertensive heart disease to systolic dysfunction [20]. To test whether myocardial structural changes were fully reversible or would progress independent of an elevated plasma homocysteine level once established, we included a group in which the hyperhomocysteinemic diet was returned to the control diet after 10 weeks and continued for 10 more weeks. This approach was utilized to avoid problems introduced by the independent effects of high-dose B-vitamin therapy detailed earlier. Our results showed that the animals that were hyperhomocysteinemic for 20 weeks developed systolic dysfunction with markedly increased myocardial interstitial fibrosis. The group that was reverted to a control diet at 10 weeks showed an intermediate phenotype, with more myocardial interstitial fibrosis than animals on the control diet, but less than animals that were hyperhomocysteinemic for 20 weeks, despite plasma homocysteine levels that had normalized. The ‘reversal’ group also showed abnormal cardiac function, which was not as severe as in the 20-week hyperhomocysteinemic animals, but worse than in animals fed a control diet for 20 weeks. These results suggest that the processes that promoted myocardial collagen deposition and myocardial dysfunction progressed even after ending the hyperhomocysteinemia-inducing diet. These data suggest that cardiac changes, once established, may progress at least partially independent of the homocysteine level. Hence, it may be important to understand the mechanisms whereby altered methionine–homocysteine metabolism modulates tissue remodeling in the cardiovascular system and target those mechanisms independent of the homocysteine level.

Could Targeting Pathogenic Mechanisms be a Better Approach Than Lowering Homocysteine Levels?

Since there are varied pathobiological mechanisms by which hyperhomocysteinemia can be disruptive (Fig. 1), targeting dominant mechanism(s) could be a novel approach to treating the cardiovascular risk attributable to hyperhomocysteinemia. A substudy of the WENBIT trial examined the effect of lowering homocysteine levels on inflammatory markers [34]. Neither combined folate/vitamin B₁₂ therapy nor vitamin B₆ therapy lowered the levels of inflammatory markers studied (soluble CD40 ligand, C-reactive protein, neopterin, or IL-6). This result suggests that homocysteine lowering with B-vitamin therapy may not always favorably affect the pathogenic milieu created by hyperhomocysteinemia.

Our laboratories have focused on oxidant stress as a major mechanism of homocysteine’s effects on vascular and cardiac tissue. GPx-1, the major cellular antioxidant enzyme that catalyzes the conversion of peroxides, including hydrogen peroxides to nontoxic molecules, is a major focus of our investigation. We have shown that homocysteine interferes with the translation of GPx-1 by a novel mechanism whereby homocysteine interferes with the incorporation of the unique selenium-containing aminoacid selenocysteine, which is crucial to the catalytic function of the enzyme [14]. The *in vivo* relevance of these findings was

assessed in mildly hyperhomocysteinemic CBS-deficient mice [35]. Mice deficient in the CBS had significantly lower GPx-1 activity. Treatment with a cysteine donor increased glutathione level and GPx-1 activity, and restored normal microvascular reactivity in CBS-deficient mice. Genetic overexpression of GPx-1 in CBS-deficient mice also normalized endothelium-dependent vasodilator response [36].

The effect of dietary antioxidant treatment was examined in our model of hyperhomocysteinemia-induced myocardial fibrosis and dysfunction [37]. Rats were fed a control or hyperhomocysteinemia-inducing diet, along with either vehicle or the combination of vitamins C and E. Antioxidant vitamin therapy did not alter plasma homocysteine levels. Notwithstanding a high homocysteine level, rats fed the antioxidant vitamin combination showed a significant decrease in myocardial oxidant stress and myocardial fibrosis, and significantly less diastolic dysfunction compared to hyperhomocysteinemic rats that did not receive antioxidant therapy. These studies suggest that therapies targeting specific pathogenic mechanisms (which could be varied in subjects with similar plasma homocysteine levels) may be a better approach than lowering homocysteine levels with therapies that may have additional deleterious effects. In this regard, a novel approach utilizing natural products that induce multiple antioxidant enzymes, such as phytochemical antioxidant stimuli, could be a better strategy compared to using antioxidants, such as vitamins C and E [38].

Could Factors That Affect Proposed Major Pathogenic Mechanisms be Crucial to “Unmasking” the Effect of Hyperhomocysteinemia?

The major pathogenic mechanisms of hyperhomocysteinemia, such as redox status and methylation status, are influenced by multiple genetic and environmental factors. Recent data suggest a significant interaction of GPx-1 and homocysteine in promoting cardiovascular risk. The AtheroGene study examined the effect of two biomarkers with “opposing” effects—homocysteine and GPx-1—on cardiovascular risk [39]. In this prospective study, 643 subjects with coronary artery disease were followed up for a median of 7.1 years. Univariate analysis adjusting for confounding variables demonstrated that homocysteine and GPx-1 were the strongest predictors of future cardiovascular events. However, combined assessment of homocysteine and GPx-1 showed that in subjects with high GPx-1 activity, a high homocysteine level did not predict future cardiovascular events. In contrast, in subjects with GPx-1 activity below the median value, plasma homocysteine levels above the median increased cardiovascular risk 3.2-fold. This study raises the interesting possibility that the pathogenic effect of hyperhomocysteinemia is significantly influenced by the overall cardiovascular redox state, specifically its antioxidant capacity as determined by GPx-1.

Dietary selenium intake is a crucial determinant of GPx-1 activity. GPx-1 belongs to a group of ~30 proteins termed selenoproteins, which require an adequate amount of selenium for expression and activity [14]. Hence, it is possible that dietary selenium intake may modulate the pathogenic effects of hyperhomocysteinemia via GPx-1 activity in cardiovascular cells. The AtheroGene investigators examined the effect of selenium supplementation on cultured human coronary endothelial cells and demonstrated that selenium supplementation increased GPx-1 expression and activity [40]. They also randomized 465 subjects with coronary artery disease and impaired endothelial function to receive two doses of sodium selenite or placebo for 12 weeks. Sodium selenite supplementation increased red blood cell GPx-1 activity compared to placebo, but did not change flow-mediated brachial artery dilation or markers of inflammation (C-reactive protein and fibrinogen), or oxidative stress (isoprostanes), over a 12-week period. This study demonstrates that selenium supplementation can be utilized to increase GPx-1 expression and activity in patients with cardiovascular disease. Further studies are needed to examine the interaction of selenium supplementation and

homocysteine metabolism, and whether selenium supplementation could be effective in countering the adverse cardiovascular effects of hyperhomocysteinemia.

Final Perspectives

Hyperhomocysteinemia is associated with cardiovascular disease; however, lowering the plasma homocysteine level utilizing high-dose B-vitamin therapy does not decrease cardiovascular risk and, therefore, is not recommended in subjects with (mild) hyperhomocysteinemia. Whether this is because hyperhomocysteinemia is only a marker of risk and not a causative factor or because B-vitamin therapy is the wrong approach to the treatment for the pathogenic mechanisms of hyperhomocysteinemia remains unclear. Because hyperhomocysteinemia denotes a perturbation of the “methionine–homocysteine axis” with varied pathogenic effects at the cellular and molecular level, B-vitamin therapy may not favorably alter the “pathogenic milieu” of hyperhomocysteinemia, and may also produce untoward effects by interference with methylation. Therapies that effectively address the consequences of hyperhomocysteinemia may offer some protection against hyperhomocysteinemia-induced cardiovascular disease, but additional research is imperative. We propose a two-pronged approach to investigation in this field. First, we endorse continued basic investigation into the precise pathogenic mechanisms and how they interact with other variables, such as redox and methylation status, to precisely understand how we can selectively abrogate each pathogenic mechanism that exists in hyperhomocysteinemic patients. The second approach is based on translational investigations using genomic and proteomic studies to understand the varied molecular mechanisms that may exist in subjects with hyperhomocysteinemia. The findings from this approach would help us to understand the mechanisms that exist in subjects with hyperhomocysteinemia, e.g., subjects with similar levels of plasma homocysteine may have predominant methylation abnormalities or altered redox status. This approach would help us understand how to select subjects with hyperhomocysteinemia for specific therapy targeting the specific mechanism or mechanisms in each subject. These approaches should cumulatively provide data that could allow us to design clinical trials that take into account the complex perturbations that exist in hyperhomocysteinemia, and allow a “personalized” approach to diagnosing and treating hyperhomocysteinemia.

Acknowledgments

This work was supported by the NIH grant R21 HL089734 (JJ).

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Fig. 1.

The methionine–homocysteine axis. Abbreviations: *MS* methionine synthase, *BHMT* betaine homocysteine methyl transferase, *B₁₂* vitamin B₁₂, *MAT* methionine adenosyl transferase, *MT* methyl transferase, *SAM* S-adenosyl methionine, *SAH* S-adenosyl homocysteine, *SAHH* S-adenosyl homocysteine hydrolase, *CBS* cystathionine beta synthase, *B₆* vitamin B₆

Table 1

Homocysteine lowering and cardiovascular disease: major recent clinical trials

Trials	No. of subjects	Inclusion criteria	Treatment groups	Effect on plasma homocysteine level ($\mu\text{mol/l}$)	Follow-up periods	Time period of study	Results
VISP [5]	3,680	Nondisabling cerebral infarction + homocysteine level	High dose Low dose	2.3 decline from baseline of 13.4 in high-dose group	2 years	1996–2003	No effect on recurrent cerebral infarction. Baseline homocysteine level was related to outcomes
HOPE-2 [6]	5,522	Age ≥ 55 years + documented vascular disease or diabetes along with other risk factors	Folate, B6, B12 Placebo	12.2 $\rightarrow$ 9.7 vs. 12.2 $\rightarrow$ 12.9	5 years	2000–2005	No effect on the combined endpoint of cardiovascular death, myocardial infarction and stroke. Regions without folate fortification had more effect with treatment
NORVIT [7]	3,749	Acute myocardial infarction ≤ 7 days	Folate, B12, B6 Folate, B12 B6 Placebo	13.1 $\rightarrow$ 9.5 12.9 $\rightarrow$ 9.8 13.3 $\rightarrow$ 13.3 13.2 $\rightarrow$ 13.6	3.5 years	1998–2004	No effect on the combined endpoint of myocardial infarction, stroke and sudden death. Increased risk in the folate + B12 + B6 group
HOST [8]	2,056	End stage renal disease or creatinine clearance ≤ 30 ml/min + homocysteine ≥ 15	Folate, B12, B6 Placebo	21.5 $\rightarrow$ 15.3 21.4 $\rightarrow$ 20.6	5 years	2001–2006	No effect on mortality in this all male cohort
WAFACS [9]	5,442	Women >40 years or post menopausal, cardiovascular disease or three risk factors	Folate, B12, B6 Placebo	12.1 $\rightarrow$ 9.8 12.5 $\rightarrow$ 11.8	7.3 years	1998–2005	No effect on the combined endpoint of myocardial infarction, stroke, coronary revascularization, cardiovascular death in this all female cohort
WENBIT [10]	3,096	Coronary angiography for suspected coronary disease or aortic stenosis	Folate, B12, B6 Folate, B12 B6 Placebo	10.8 $\rightarrow$ 7.6 in first two groups with folate and B12 No change in B6 or placebo groups	38 months	1999–2006	No effect on composite endpoint of total mortality, nonfatal myocardial infarction, hospitalization for unstable angina, or nonfatal thromboembolic stroke

Folate fortification started in North America in 1996, was mandated in 1998