REVIEW ARTICLE

Drugs 2002; 62 (4): 605-616
0012-6667/02/0004-0605/$30.00/0

Drugs Affecting Homocysteine Metabolism

Impact on Cardiovascular Risk

Cyrus Desouza, Mary Keebler, Dennis B. McNamara and *Vivian Fonseca*

Tulane University School of Medicine, New Orleans, USA

Contents

Abstract 605
1. Homocysteine Metabolism 606
2. Premature Vascular Disease and Hyperhomocysteinaemia 607
3. Drugs and Homocysteine 608
   3.1 Lipid Lowering Drugs 608
   3.2 Metformin 610
   3.3 Insulin 610
   3.4 Sex Hormones 610
      3.4.1 Estrogen 611
      3.4.2 Testosterone 611
   3.5 Drugs Used in Rheumatoid Arthritis 611
   3.6 Antiepileptic Drugs 612
4. Management of Hyperhomocysteinaemia 612
   4.1 Prevention 612
   4.2 Treatment 612
      4.2.1 Betaine 613
5. Effect of Treatment on Cardiovascular Disease and Surrogate Outcomes 613
6. Conclusion 614

Abstract

Elevated total plasma homocysteine has been established as an independent risk factor for thrombosis and cardiovascular disease. A strong relationship between plasma homocysteine levels and mortality has been reported in patients with angiographically confirmed coronary artery disease.

Homocysteine is a thiol containing amino acid. It can be metabolised by different pathways, requiring various enzymes such as cystathionine β-synthase and methylenetetrahydrofolate reductase. These reactions also require several co-factors such as vitamin B6 and folate. Medications may interfere with these pathways leading to an alteration of plasma homocysteine levels.

Several drugs have been shown to effect homocysteine levels. Some drugs frequently used in patients at risk of cardiovascular disease, such as the fibric acid derivatives used in certain dyslipidaemias and metformin in type 2 (non-insulin–dependent) diabetes mellitus, also raise plasma homocysteine levels. This elevation poses a theoretical risk of negating some of the benefits of these drugs.

The mechanisms by which drugs alter plasma homocysteine levels vary. Drugs

such as cholestyramine and metformin interfere with vitamin absorption from the gut. Interference with folate and homocysteine metabolism by methotrexate, nicotinic acid (niacin) and fibric acid derivatives, may lead to increased plasma homocysteine levels.

Treatment with folate or vitamins B6 and B12 lowers plasma homocysteine levels effectively and is relatively inexpensive. Although it still remains to be demonstrated that lowering plasma homocysteine levels reduces cardiovascular morbidity, surrogate markers for cardiovascular disease have been shown to improve with treatment of hyperhomocystenaemia. Would drugs like metformin, fibric acid derivatives and nicotinic acid be more effective in lowering cardiovascular morbidity and mortality, if the accompanying hyperhomocysteinaemia is treated? The purpose of this review is to highlight the importance of homocysteine as a risk factor, and examine the role and implications of drug induced modulation of homocysteine metabolism.

Homocysteine is a thiol containing amino acid formed by demethylation of methionine. Elevated plasma homocysteine levels have been established as an independent risk factor for thrombosis and vascular disease. Several epidemiological studies have shown that moderately elevated plasma homocysteine levels are associated with an increased risk for fatal and non-fatal cardiovascular disease.[1-4] Although simple inexpensive treatment with folic acid, vitamin B6 (pyridoxine) and vitamin B12 (cyanocobalamin) is highly effective in lowering plasma homocysteine levels, it still remains to be demonstrated that lowering levels of homocysteine reduces cardiovascular morbidity and mortality.[1]

Several drugs have been shown to modulate the increased plasma homocysteine levels. They act via different mechanisms including inhibition of vitamin absorption, altering homocysteine metabolism, interfering with renal function and influencing hormonal status.[2] Some drugs, frequently used in patients at risk of cardiovascular disease, such as fibric acid derivatives and metformin also increase plasma homocysteine levels. This elevation poses a theoretical risk of negating some of the benefits of these drugs. Would fibric acid derivatives be more effective in lowering the incidence of cardiovascular disease if the accompanying hyperhomocysteinaemia was treated? Would metformin decrease cardiovascular disease further in patients with diabetes mellitus if hyperhomocysteinaemia was treated or prevented? The answer is not known and further studies are needed to determine the mechanism of action of these changes and whether they have a negative impact. As long as potential atherogenicity and thrombogenicity of raised plasma homocysteine levels remains a concern, simple, effective and non-toxic treatment with folic acid, and vitamins B6 and B12 may be a reasonable option. The purpose of this review is to summarise the metabolism of homocysteine and its importance as a cardiovascular risk factor, and then to highlight the role of drug therapy in altering homocysteine levels.

1. Homocysteine Metabolism

Homocysteine is a sulphur containing amino acid, formed by demethylation of methionine. Homocysteine can be metabolised by two major pathways. When methionine is in excess, homocysteine is metabolised via the trans-sulfuration pathway. Homocysteine irreversibly condenses with serine to form cystathionine. This reaction is catabolised by cystathionine β-synthase (CBS) in a process requiring vitamin B6 as a cofactor. Cystathionine is then hydrolysed to cysteine by the enzyme cystathioninase where vitamin B6 is also a cofactor. Under conditions of negative methionine balance, homocysteine is primarily metabolised via the remethylation pathway. Remethylation may occur by one of two reactions. In one, homocysteine is reconverted to methionine by transfer of a methyl

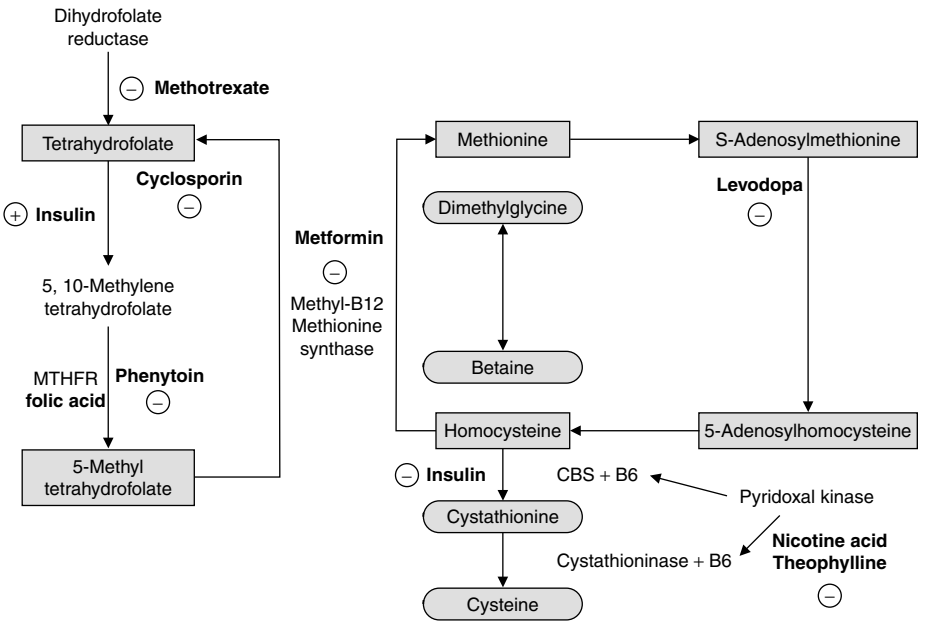

Fig. 1. A simplified pathway of homocysteine metabolism with known sites of interference by drugs indicated. **B6** = vitamin B6; **B12** = vitamin B12; **CBS** = cystathionine β-synthase; **MTHFR** = methylenetetrahydrofolate; **+** indicates stimulation of enzyme or pathway; **–** indicates inhibition of enzyme or pathway.

group from 5-methyltetrahydrofolate in a reaction catalysed by cobalamine-dependant methionine synthase. The formation of 5-methyl tetrahydrofolate is catalysed by methylenetetrahydrofolate reductase (MTHFR) which requires riboflavin as a cofactor. The other remethylation pathway uses betaine as a methyl donor and requires betaine-homocysteine methyltransferase (BHMT).[5]

In plasma, approximately 70% of homocysteine circulates in a protein bound form and 25% combines to form a homocysteine dimer. The remainder combines with other thiols, including cysteine, to form disulphide or circulates as the free thiol compound. Total homocysteine encompasses all the above components and is frequently abbreviated as tHcy.

Figure 1 illustrates a simplified pathway of homocysteine metabolism with known sites of interference by drugs.

2. Premature Vascular Disease and Hyperhomocysteinaemia

Several studies have attempted to establish the prevalence of hyperhomocysteinaemia in patients with premature and accelerated vascular disease.[1]

Boers et al.[6] drew attention to elevated plasma homocysteine levels following a methionine load in 28% of patients with peripheral vascular and cerebrovascular disease. Even after adjustment for other risk factors, plasma homocysteine has been found to be significantly higher in patients with peripheral vascular disease compared with healthy individuals. Elevations in peak homocysteine following a methionine load occur in 28 to 42% of patients with vascular disease but rarely if ever in healthy individuals.[7]

In a meta-analysis of 27 studies of homocysteine in atherosclerotic vascular disease, Boushey et al.[8] concluded that elevations of homocysteine levels were an independent risk factor for arterio-

sclerosis. They estimated that approximately 10% of the population's coronary artery disease risk appears attributable to elevated homocysteine levels, and that an increase in plasma homocysteine increased the odds ratio for coronary artery disease by 1.6 for men and 1.8 for women.[8]

A strong relationship between plasma tHcy and mortality has been reported in patients with angiographically confirmed coronary artery disease. Less than 4% of patients with a plasma tHcy below 9 μmol/L died, compared with nearly 25% of those with a level greater than 15 μmol/L.[9]

Omland et al.[10] recently reported that patients with acute coronary syndromes whose serum homocysteine level on admission was >14.1 μmol/L had a relative risk of 1.7 for all causes of death, compared with those with a lower plasma homocysteine level. This suggests a possible prognostic value to measuring plasma homocysteine levels.

Hyperhomocysteinaemia appears to have its strongest association with carotid artery disease and stroke. In the Framingham Heart Study, a 2-fold increase in the incidence of carotid disease was seen in patients with the highest plasma homocysteine levels when compared to those with the lowest levels.[11,12]

In summary, data from many studies support the hypothesis that hyperhomocysteinaemia is an independent risk factor for coronary artery disease, as well as other arterial occlusive disease.[13,14]

3. Drugs and Homocysteine

As mentioned in section 1, several drugs alter homocysteine concentration. Table I lists the drugs known to affect plasma homocysteine levels and the possible mechanisms involved. The drugs we consider important in clinical practice are discussed below.

3.1 Lipid Lowering Drugs

Lipid lowering drugs are clearly effective in lowering the rate of events in coronary heart disease. Lipid lowering agents not only alter plasma lipid levels, but also have other metabolic effects such as decreasing plasma fibrinogen levels and improving endothelial dysfunction. Some of these effects may potentially and paradoxically increase cardiovascular risk. Fibric acid derivatives, cholestyramine and nicotinic acid (niacin) have all been shown to increase plasma homocysteine.[15]

De Lorgeril and colleagues[16] reported a 46% increase in plasma homocysteine in patients who were treated with fenofibrate for 12 weeks. Dierkes and colleagues demonstrated a 44 and 17.5% increase in homocysteine levels after treatment with fenofibrate and bezafibrate, respectively. Vitamin levels remained unaffected through the study.[15] This suggests that the mechanism of increased homocysteine levels is not vitamin dependent. Fibric acid derivatives have frequently been reported to increase serum creatinine moderately and reversibly. This may reflect a functional reduction of glomerular filtration rate and hence explain increased homocysteine levels.[15]

Recent epidemiological data, such as the Veterans Administration HDL (high density lipoprotein)-Cholesterol Interventional Trial and the St. Mary's, Northwick Park Diabetes CVD (cardiovascular disease) Prevention Study have shown decrease in cardiovascular events in patients treated with gemfibrozil and bezafibrate.[17,18] Some studies do not show this benefit.[19] Thus data on the benefits of fibric acid derivatives is conflicting. It is therefore important to determine whether the increase in homocysteine negates the benefit of these agents on lipids.[20,21] In contrast, HMG-CoA reductase inhibitors, which have no significant effects on homocysteine levels, show a clear and consistent benefit in lowering cardiovascular mortality.[22,23]

Interference with cobalamine absorption thus leading to a slow increase in plasma homocysteine has been reported with cholestyramine.[24] In addition, elevation of homocysteine following treatment with cholestyramine is largely confined to individuals with C677T mutation in the *MTHFR* gene.[25]

Nicotinic acid in large doses has been used frequently for its lipid lowering effects. However the potential risks involved need to be carefully con-

Table I. Effects of drugs on plasma homocysteine levels

Drug class/drug	Effect on homocysteine levels	Mechanism of effect
Lipid lowering drugs		
Fibric acid derivatives (e.g. fenofibrate)	Increases	Not known? Functional reduction of GFR?
Nicotinic acid	Increases	Inhibition of pyridoxal kinase, decreased vitamin B6 levels, decreased activity of CBS
Cholestyramine	Increases	Interference with vitamin B12 and folate absorption
HMG-CoA reductase inhibitors	No effect	
Diabetes drugs		
Metformin	Increases	Interference with vitamin B12, depression of intrinsic factor secretion, binding of free calcium in the gut, possible folate lowering effect
Insulin	Decreases	Increased activity of MTHFR, decreased activity of CBS
Sex hormones		
Estrogens	Decreases	Mechanism not clear
Androgens/testosterone	Increases	Increased creatinine synthesis, differences in sex steroid milieu
Anti-estrogens		
Tamoxifen/raloxifene	Decreases	Modest elevations in folate levels, estrogen receptor mediated?
Anti-rheumatic drugs		
Methotrexate	Increases	Inhibition of dihydrofolate reductase
Sulfasalazine	Small non-sustained acute rise	Mechanism not clear
Anti-epileptic drugs		
Phenytoin	Increase	Folate depletion, possible decreased activity of 5-MTHFR, decreased activity of methionine synthase, hepatic enzyme induction
Carbamazepine	Increase	Folate depletion, possible hepatic enzyme induction
Valproic acid	Non-significant effect	
Other drugs		
Cyclosporin	Increases	Interference with renal function, possible interference in folate dependant remethylation
Theophylline	Increases	Pyridoxal kinase inhibition
Levodopa	Increases	Acts as a substrate for S-adenosylmethionine dependant transmethylation
Acetylcysteine	Decreases	Thiol-disulfide exchange, lower plasma protein binding
Alcohol	Increases	Possible depletion of folate

CBS = cystathionine β-synthase; **GFR** = glomerular filtration rate; **MTHFR** = methyltetrahydrofolate reductase.

sidered. Nicotinic acid interferes with the metabolism of methionine, leading to hyperhomocysteinaemia. Nicotinic acid may raise plasma homocysteine levels as a result of the inhibition of pyridoxal kinase. Inhibition of pyridoxal kinase results in decreased pyridoxine levels, which is an important co-factor for CBS which catabolises homocysteine to cystathionine.[26] Whether this elevation of homocysteine has any clinical significance has still not been studied adequately.

These findings may be important in view of the well-documented association of even moderate elevation of tHcy with arthrosclerotic manifestations. These studies also have important clinical implications in the management of patients with diabetic dyslipidaemia, for which drugs like metformin and fibric acid derivatives are frequently used. Munshi et al.[27] reported that hyperhomocysteinaemia after a methionine load occurred in approximately 40% of patients with type 2 (non-insulin–dependent) diabetes mellitus. If hyperhomocysteinaemia ensues, it may be reasonable to treat these elevations of homocysteine with vitamin therapy. However, clinical trials have

not been carried out and vitamin therapy fails to correct many cardiovascular risk factors frequently abnormal in patients with type 2 diabetes.[28]

3.2 Metformin

Patients with type 2 diabetes are very often treated with metformin which is a first line drug for obese patients. This is because metformin favourably modifies insulin resistance by decreasing glucose production by the liver and increasing peripheral uptake of glucose. Metformin may also have positive effects on cardiovascular risk factors associated with type 2 diabetes. Metformin monotherapy decreased cardiovascular mortality in the UK Prospective Diabetes study (UKPDS).[29] Several other studies including the Biguanides and Prevention of Risks in Obesity (BIGPRO) trial and the Diabetes and Thrombosis research group, UK, have shown that metformin tends to stabilise or decrease weight, has beneficial effects of plasma lipids, and may also improve blood pressure and enhance fibrinolysis.[30,31]

On the other hand, metformin has been shown to increase total serial homocysteine levels in several studies. A rise in plasma homocysteine levels in people with diabetes may have important clinical implications, as suggested by data from case-control and epidemiological studies.[32] Carlson et al. showed that homocysteine levels in non-diabetic male patients with coronary heart disease, increased moderately by 7.2 and 13.8% after 12 and 40 weeks of therapy, respectively.[33]

There are two different mechanisms by which metformin increases homocysteine levels. Metformin is known to decrease serum vitamin B12 levels.[30,31] This is possibly mediated by changes in intestinal bacterial flora, that increase microbacterial intrinsic factor utilisation.[30,31] Depression of intrinsic factor secretion has also been implicated. Metformin may also bind free calcium which is required for the uptake of the vitamin B12-intrinsic factor complex in the ileum by its receptor.[34] Bauman et al.[34] showed that calcium supplementation reversed this effect. Thus, metformin may cause malabsorption of vitamin B12 by various mechanisms.

3.3 Insulin

There are few studies showing the relationship between plasma homocysteine and endogenous insulin levels in patients with type 2 diabetes. Fonseca et al.[35] noted that hyperinsulinaemia results in decreased homocysteine levels in healthy volunteers but not in patients with insulin resistant type 2 diabetes. Drzewoski et al.[36] found that long-term poor metabolic control of type 2 diabetes was characterised by elevations of plasma homocysteine and this was inversely correlated with endogenous insulin levels. Cronin et al.[37] noted lower homocysteine levels in patients with well-controlled type 1 (insulin-dependent) diabetes mellitus. Hultberg et al.[38]showed that deterioration of metabolic control in patients with type 1 diabetes resulted in increased homocysteine levels.

Insulin has powerful effects on protein and amino acid metabolism and decreases plasma methionine levels. Jacobs et al.[39] investigated homocysteine metabolism in a type 1 diabetic animal model (streptozotocin-treated rats) to examine whether insulin plays a role in its regulation. Plasma homocysteine levels were lower in untreated diabetic rats but this decrease in homocysteine was prevented when the diabetic rats received insulin. They observed an effect of insulin in the activities of the hepatic transsulfuration enzymes.

Rats fed with a high fat-sucrose diet had elevated plasma insulin and homocysteine levels, associated with a decreased hepatic CBS enzyme activity and increased hepatic MTHFR enzyme activity. The degree of activity of these two enzymes varied with the degree of hyperinsulinaemia.[40]

3.4 Sex Hormones

Men have an increased risk of developing coronary vascular disease and so do postmenopausal women. After menopause the risk of cardiovascular disease in women rapidly increases. The reasons for this increase are likely to be multifactorial. An

elevated plasma homocysteine level is an independent risk factor for cardiovascular disease. A sex difference in tHcy has been found with an approximately 10 to 15% higher level in women. There is considerable data that sex hormones affect plasma homocysteine levels and may explain the increase cardiovascular disease in men and postmenopausal women.

3.4.1 Estrogen

The mechanism by which estrogen has an effect on homocysteine metabolism is not clear. Compared with non pregnant women, the state characterised by high levels of endogenous estrogen, tHcy was significantly decreased in pregnant women.[41] Wouters et al.[42] showed that fasting and post methionine plasma homocysteine levels were significantly higher in postmenopausal women than in premenopausal women. Furthermore, in postmenopausal women treatment with oral 17β-estradiol (estradiol) or conjugated estrogens resulted in a decrease of plasma tHcy by 11% after 6 months. Giltay et al.[43] found a significant reduction in plasma tHcy male transsexuals after estrogen and anti-androgen therapy. The postmenopausal Estrogen/Progestin Interventions (PEPI) trial also suggested that estrogen therapy lowered plasma homocysteine levels in postmenopausal women with normal homocysteine levels at baseline.[44] There are several other studies confirming the same, although one study found no relationship between estrogen and homocysteine.[45-47]

Oral contraceptive agents in contrast to hormone replacement therapy have no significant impact on homocysteine levels in young women.[48] Tamoxifen, an estrogen antagonist with partial agonist effects, significantly decreased plasma homocysteine levels by 30% after 9 to 12 months treatment in postmenopausal women with breast cancer.[49] In addition to its estrogen receptor mediated effects, tamoxifen may be associated with modest elevations in plasma folate levels. Walsh et al.[50] showed that raloxifene also lowers plasma homocysteine levels in postmenopausal women.

3.4.2 Testosterone

Zmunda et al.[51] studied the effect of supra physiological doses of testosterone on fasting homocysteine levels in normal weight lifters. Plasma homocysteine levels were not significantly altered where testosterone was given alone or together with the testolactone. Therefore, it is likely that short-term high-dose testosterone does not effect plasma homocysteine levels. In contrast, in a study of female to male transsexuals, there was a significant increase in plasma tHcy after testosterone administration.[43] This may be explained by greater creatinine synthesis and larger muscle mass in men compared with women. An explanation for this could be that homocysteine production occurs in direct conjunction with creatine-creatinine synthesis.[43] The sex difference in plasma tHcy may thus seem related to their differences in sex steroid milieu.

3.5 Drugs Used in Rheumatoid Arthritis

There is increasing evidence that patients with rheumatoid arthritis have increased mortality from cardiovascular events. Mylljkangas and colleagues[52] found that 50% of deaths in women with rheumatoid arthritis in their study were due to cardiovascular events. Wallberg-Johsson et al.[53] showed increased overall mortality and death due to cardiovascular disease in sero-positive rheumatoid arthritis patients. Certain disease modifying antirheumatic drugs such as methotrexate and sulfasalazine have been known to increase homocysteine levels. It is not known whether homocysteine is a risk factor for cardiovascular disease in patients with rheumatoid arthritis. However, elevated plasma homocysteine levels occur commonly in patients with rheumatoid arthritis and may explain some of the increased cardiovascular mortality seen in these patients.[54] It may be prudent to monitor plasma levels of this risk factor in patients receiving these drugs.

In a study by Haagsme et al.,[55] patients with rheumatoid arthritis receiving sulfasalazine showed only a small acute rise in plasma homocysteine levels which returned to normal quickly. Patients re-

ceiving methotrexate had a clear rise in plasma homocysteine levels, which remained increased up to 6 months of treatment. Methotrexate also induces increased homocysteine levels when used for cancer chemotherapy. Methotrexate is a well known anti-metabolite, with dihydrofolate reductase inhibition as the primary target, which results in depletion of reduced folates and hence hyperhomocysteinaemia. This is further supported by the fact that folinic acid rescue reverses the hyperhomocysteinaemia.[55] Thus, the effects of methotrexate on plasma homocysteine are clearly linked with its effect on folate metabolism.[56]

Therefore, because of the association of hyperhomocysteinaemia with arthrosclerosis, it would be worthwhile lowering homocysteine levels with folates in patients receiving methotrexate therapy. Folic acid improves folate status and prevents the methotrexate-induced hyperhomocysteinaemia and toxicity, while preserving therapeutic efficacy in patients with rheumatoid arthritis.[56]

3.6 Antiepileptic Drugs

Antiepileptic drugs (AED) are well known to induce folate deficiency. This predisposes the patients receiving certain AED to have elevated plasma homocysteine levels. Folic acid is required for remethylation of homocysteine to methionine. A deficiency of folate results in impaired remethylation, methionine depletion and hence, accumulation of homocysteine. The mechanism of folate depletion is not clear. Some studies have shown decreased activity of 5-MTHFR and methionine synthase.[57,58] Folate deficiency may also be due to hepatic enzyme induction by some AED such as carbamazepine.

Several studies have implicated AED as elevating homocysteine levels. Hiroaki Ono et al.[57] demonstrated raised homocysteine and lower folic acid levels in patients receiving AED. In a study of patients receiving carbamazepine, phenytoin or phenobarbital, Schwaninger et al.[58] showed that plasma homocysteine levels were significantly increased by 14.7% in patients versus controls. Valproic acid on the other hand is associated with only a small risk of folate deficiency.[59]

As homocysteine is an established risk factor in arthrosclerosis, this becomes an issue of concern in patients on long-term antiepileptic therapy, although it is not known whether there is an increased risk for cardiovascular disease in patients receiving AED. Importantly, homocysteine is itself a potential convulsant, and may reduce seizure threshold and increase seizure frequency in these patients.[57,58]

4. Management of Hyperhomocysteinaemia

Several agents are known to decrease plasma homocysteine levels. Most of them are inexpensive and have good safety profiles. There have been several trials showing that the surrogate markers of cardiovascular disease improve with treatment of hyperhomocysteinaemia. Whether this translates into improved cardiovascular outcomes is still under investigation.

4.1 Prevention

Fortification of foods with folic acid and vitamins B6 and B12 has been studied. Schorah et al.[60] carried out a randomised, double-blind, placebo-controlled trial in 119 volunteers, whose intake of fortified or supplemental folic acid was low. Folic acid supplementation of cereals led to significant increases in serum folate (66%) and decrease in plasma homocysteine (10%) levels.

Clinical trials have not shown any benefit in terms of lowering homocysteine levels when healthy volunteers received pyridoxine or cyanocobalamin.[61] The impact of increased nutritional supplementation with folic acid on homocysteine levels in the general population needs to be further evaluated.

4.2 Treatment

Folic acid and vitamins B6 and B12 have been used in various studies with some success in lowering homocysteine levels. There is no consensus

on the optimal dosage of vitamins to be used in the treatment of hyperhomocysteinaemia. There is as yet little evidence that lowering homocysteine levels has any effect on lowering the risk of cardiovascular disease and this presents a dilemma to the practising physician.

Combination therapy with different vitamins may be necessary to achieve adequate suppression of homocysteine levels in many patients. Franken et al.[62] treated patients with mild hyperhomocysteinaemia with pyridoxine 250 mg/day for 6 weeks after which the post-load plasma homocysteine level fell significantly in 56% of the patients. Further treatment with the addition of folic acid and/or betaine hydrochloride resulted in normalisation of homocysteine concentration in 95% of the remaining patients.

Only half of patients with CBS deficiency respond to pyridoxine. This may be because some non-responders may have folate deficiency (which can block the response to pyridoxine until folate is replaced) or decreased affinity of the mutant enzyme for the cofactor.[63-65] Van den Berg et al.[66] have also demonstrated correction of mild hyperhomocysteinaemia in a subset of young patients with cardiovascular disease. Brattstrom et al.[67] demonstrated that pyridoxine 240 mg/day plus folic acid 10 mg/day reduced fasting homocysteine levels by a mean of 53% and a post-methionine load homocysteine level by a mean of 39%.

Ubbink and colleagues[68] investigated the roles of three vitamins as determinants of plasma homocysteine levels in a placebo-controlled study. One hundred individuals with high fasting plasma homocysteine levels were enrolled in the trial, which compared treatment with five different regimens: 1) placebo; 2) folic acid 0.65mg; 3) pyridoxine 10 mg; 4) cyanocobalamin 0.4 mg; and 5) a combination of all three vitamins. Plasma homocysteine levels were assayed 4 and 6 weeks after starting vitamin therapy. Folic acid lowered plasma homocysteine by 42% and cyanocobalamin by only 15%. Pyridoxine had no significant effect as it lowers post-methionine homocysteine levels rather than fasting levels. The combination of three vitamins did not differ from the effect of folic acid alone.

Boer et al.[69] treated 32 patients with post-methionine load hyperhomocysteinaemia with pyridoxine 250 mg/day for 6 weeks. A few patients also received folic acid 5 mg/day in addition (if they were folate deficient). 81% of patients responded with the normalisation of post-load hyperhomocysteinaemia. Similarly Brattstrom et al.[67] reported a 26% reduction in post-load plasma homocysteine levels in patients receiving pyridoxine 15 mg/day and a further significant reduction of up to 39% when folic acid 10 mg/day was added.

Treatment with cyanocobalamin does not appear to have a very significant effect on plasma homocysteine levels in healthy individuals but may reduce it in some patients with hyperhomocysteinaemia, particularly those who have low or normal blood vitamin B12 levels.

In summary, although no consensus exists it seems likely that folic acid 0.65mg daily may be sufficient to lower mild hyperhomocysteinaemia significantly. However, in patients who have a methionine load test (MLT) and are found to have an elevated post-load homocysteine level, treatment with pyridoxine is necessary. The appropriate dose of pyridoxine has not been determined, although up to 250 mg/day has been used in several studies.[62,67]

4.2.1 Betaine

Betaine is a methyl group donor involved in the metabolism of methionine. This remethylation pathway operates independently of vitamin B12 and folate, but uses betaine as a methyl donor and requires the enzyme BNMT.

Therefore, betaine-dependant remethylation is an important pathway which lowers homocysteine levels independent of the B vitamins.[70]

5. Effect of Treatment on Cardiovascular Disease and Surrogate Outcomes

There are currently no published data on the efficacy of tHcy lowering therapy on cardiovascular events. However, there are on-going clinical trials to test this hypothesis. Until these trials are com-

plete we have only studies on surrogate outcomes on which to draw conclusions. Large-scale clinical trials take a long time to complete, and are sometimes limited in their design and conclusions. Surrogate markers may provide us with reassurance that we are at least not doing our patients harm when recommending tHcy lowering therapy. Some of these markers, such as endothelial function, have been shown to be associated with clinical outcomes with other treatments.[71-74]

Vermeulen et al.[75] recently published results from a randomised, placebo-controlled trial of 158 healthy siblings of 167 patients with premature cardiovascular disease. Eighty patients were given placebo, while 78 patients adhered to a vitamin regimen consisting of pyridoxine 250 mg/day and folic acid 5 mg/day. The patients were followed over 2 years of treatment for development or progression of sub-clinical atherosclerosis. The patients who took vitamins had decreased pre- and post-MLT plasma homocysteine levels and a decreased rate of abnormal exercise electrocardiographic findings compared with the controls.

Arterial endothelial dysfunction is an important early event in atherogenesis. Several studies have shown endothelial dysfunction associated with increased homocysteine levels.[73,74] As endothelial dysfunction and increased thrombogenicity seem to be the two mediators of increased cardiovascular risk in patients with hyperhomocysteinaemia, it would be logical to see if treatment with folic acid plus pyridoxine not only reduced homocysteine levels but also improved endothelial dysfunction and decreased thrombogenicity. Several studies have tried to assess improvement in endothelial dysfunction by measuring changes in flow mediated dilatation in the brachial artery.[71,76] Other studies have focused on markers of coagulation and fibrinolysis such as prothrombin fragments 1 and 2, thrombomodulin, fibrinopeptide a, plasminogen activator inhibitor-1, intercellular adhesion molecules 1, and von Willebrand factor.[73,77] Results of these studies are promising except in patients receiving dialysis. Further studies are needed to determine whether the improvement in surrogate risk markers will lead to a reduction in clinical events and mortality.

6. Conclusion

We conclude that drug interactions with homocysteine metabolism are an important determinant of plasma homocysteine levels. These effects must be considered when evaluating a patient who has an increased plasma homocysteine level. Whether these effects of drugs lead to changes in risk of cardiovascular disease requires further investigation.

Acknowledgements

Research in Diabetes, Vascular Disease and Homocysteine metabolism at Tulane University Health Sciences Center is supported in part by the Tullis-Tulane Alumni Chair in Diabetes and the John C. Cudd Fund. Research in homocysteine metabolism is funded in part by grant-in-aid from American Heart Association, southeastern affiliate.

References

1. Eikelboom JW, Lonn E, Genest J, et al. Homocysteine and cardiovascular disease: a critical review of the epidemiological evidence. Ann Intern Med 1999; 131 (5): 363-75
2. Niranjan S, Killian R. Risk factor modification for cardiac disease: homocysteine, B-vitamins, and coronary artery disease. Med Clin North Am 2000; 84 (1): 215-37
3. Refsum H, Ueland PM, Nygard O, et al. Homocysteine and cardiovascular disease. Annu Rev Med 1998; 49: 31-62
4. Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary arteriosclerosis. J Am Coll Cardiol 1996; 27: 517-27
5. Fonseca V, Guba SC, Fink LM. Hyperhomocystenemia and the endocrine system: implications for atherosclerosis and thrombosis. Endocr Rev 1999; 20 (5): 738-59
6. Boers GH, Smals AG, Trijbels F, et al. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med 1985; 313: 709-15
7. Bostom AG, Jacques PF, Nadeau MR, et al. Post-methionine load hyperhomocystenemia in persons with normal fasting total plasma homocysteine: the NHLBI family heart study. Atherosclerosis 1995; 116: 147-51
8. Boushey CJ, Beresford SA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA 1995; 274: 1049-57
9. Nygard O, Nordrehaug JE, Refsum H, et al. Plasma homocysteine concentration and mortality in patients with coronary artery disease. N Engl J Med 1997; 337: 230-6
10. Omland T, Samuelsson A, Hartford M, et al. Serum homocysteine concentration as an indicator of survival in patients with acute coronary syndromes. Arch Intern Med 2000; 160: 1834-40
11. Selhub J, Jacques PR, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 1995; 332: 286-91

12. Aronow WS, Ahn C, Schoenfeld MR. Association between plasma homocysteine and extracranial carotid arterial disease in older persons. Am J Cardiol 1997; 79: 1432-3
13. Konecky N, Malinow MR, Tunick PA, et al. Correlation between plasma homocyst(e)ine and aortic atherosclerosis. Am Heart J 1997; 133: 534-40
14. Bots ML, Launer LJ, Lindemans J, et al. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam study. Arch Intern Med 1999; 159: 38-44
15. Dierkes J, Westphal S, Luley C. Serum homocysteine increases after therapy with fenofibrate or bezafibrate [abstract]. Lancet 1999; 354: 219-20
16. De Logeril M, Salen O, Paillard F, et al. Lipid-lowering drugs and homocysteine [abstract]. Lancet 1999; 353: 209-10
17. Rubins HB, Robins SJ, Collins D, et al. Gemfibrosal for the secondary prevention of coronary heart disease in men with low concentration of HDL cholesterol. Veterans affairs HDL-cholesterol intervention trial study group. N Engl J Med 1999; 314 (6): 410-8
18. Elkeles RS, Diamond JR, Poulter C, et al. Cardiovascular outcomes in type 2 diabetes. A double-blind placebo-controlled study of Bezafibrate: the St Mary's Ealing, Northwick Park diabetes cardiovascular disease prevention (SENDCAP) study. Diabetes Care 1998; 21 (4): 641-8
19. Frick MH, Heinonen OP, Huttunen JK, et al. Efficacy of gemfibrozil in dyslipidemic subjects with suspected heart disease: an ancillary study in the Helsinki heart study frame population. Ann Med 1993; 25 (1): 41-5
20. Jonkers IJAM, De Man FHAH, Onkenhout W, et al. Implication of fibrate therapy for homocysteine [abstract]. Lancet 1999; 354: 1208
21. Watts GF, Dimmitt SB. Fibrates, dyslipoproteinemia and cardiovascular disease. Lipidology 1999; 10 (6): 561-74
22. Bucher HC, Griffith LE, Guyatt GH. Systematic review on risk and benefit of different cholesterol lowering interventions. Arterioscler Thromb Vasc Biol 1999; 19 (2): 187-95
23. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary artery disease: the Scandinavian simvastatin survival study (4S). Lancet 1994; 344: 1383-9
24. Coronato A, Glass GB. Depression of intestinal uptake of radiovitamin B12 by cholestyramine. Proc Soc Exp Biol Med 1973; 142: 1341-4
25. Tonstad S, Refsum H, Ose L, et al. The C677T mutation in MHTFR gene predisposes to hyperhomocystenemia in children with familial hypercholestremia treated with cholestyramine. J Pediatr 1998; 132: 365-8
26. Basu TK, Mann S. Vitamin B6 normalizes the altered sulphur aminoacid status of rats fed diets containing pharmacological concentration of niacin without reducing niacins hypolipidemic effect. J Nutr 1997; 127: 117-21
27. Munshi MN, Stone A, Fink L, et al. Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. Metabolism 1996; 45: 133-5
28. Baliga BS, Reynolds T, Fink LM, et al. Hyperhomocysteinemia in type 2 diabetes mellitus: cardiovascular risk factors and effect of treatment with folic acid and pyridoxine. Endocr Pract 2000; 6 (6): 435-41
29. Effect of blood glucose control with metformin on complications in overweight patients with type 2 diabetes. UKPDS Group. Lancet 1998; 352: 854-65
30. Fontbonne A, Charles MA, et al. The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution: the biguanides and prevention of rRisks in obesity(BIGPRO) study group. Diabetes Care 1996; 19 (9): 920-6
31. Grant PJ. The effects of high and medium dose metformin therapy on cardiovascular risk factors in patients with type 2 diabetes: the diabetes and thrombosis research group. Diabetes Care 1996; 19 (1): 64-6
32. Palumbo PJ. Metformin: effects on cardiovascular risk factors in patients with non-insulin-dependent diabetes mellitus. J Diabet Complications 1998; 12 (2): 110-9
33. Carlsen SM, Folling I, Grill V, et al. Metformin increases total serum homocysteine concentration in non-diabetic male patients with coronary artery disease. Scand J Clin Lab Invest 1997; 57: 521-8
34. Bauman WA, Shaw S, Jayatilleke E, et al. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care 2000; 23 (9): 1227-31
35. Fonseca VA, Mudaliar S, Schmidt B, et al. Plasma homocysteine concentrations are regulated by acute hyperinsulinemia in non-diabetic but not type 2 diabetic subjects. Metabolism 1998; 47: 686-9
36. Drzewoski J, Czupryniak L, Chwatko G, et al. Total homocysteine and insulin concentration in type 2 diabetic patients with secondary failure to oral agents. Diabetes Care 1999; 22 (12): 2097-9
37. Cronin CC, McPartlin JM, Barry DG, et al. Plasma homocysteine concentrations in patients with type 1 diabetes. Diabetes Care 1998; 21: 1843-7
38. Hultberg B, Agardh CD, Agardh E, et al. Poor metabolic control, early age at onset, and marginal folate deficiency are associated with increasing concentration of plasma homocysteine in insulin dependant diabetes mellitus: a 5 year follow up study. Scan J Clin Lab Invest 1997; 57: 595-600
39. Jacobs RL, House JD, Brosnan ME, et al. Effects of streptozotocin-induced diabetes and of insulin treatment on homocysteine metabolism in the rat. Diabetes 1998; 47: 1967-70
40. Fonseca V, Dicker-Brown A, Ranganathan S, et al. Effects of a high fat-sucrose diet on enzymes in homocysteine metabolism in the rat. Metabolism 2000; 49 (6): 736-41
41. Andersson A, Hultberg B, Brattstrom L, et al. Decreased serum homocysteine in pregnancy. Eur J Clin Chem Biochem 1992; 30: 377-9
42. Wouters MG, Moorrees MT, van der Mooren MJ, et al. Plasma homocysteine and menopausal status. Eur J Clin Invest 1995; 25: 801-5
43. Giltay EJ, Hoogeveen EK, Elbers JMH, et al. Effects of sex steroids on plasma total homocysteine concentration: a study in transsexual males and females. J Clin Endocrinol Metab 1998; 83 (2): 550-3
44. Barnabei VM, Phillips TM, Hsia J. Plasma homocysteine in women taking hormone replacement therapy: the postmenopausal estrogen/progestin interventions(PEPI) trial. J Womens Health Gend Based Med 1999; 8 (9): 1167-72
45. Hak AE, Polderman KH, Westendorp IC, et al. Increased plasma homocysteine after menopause. Atherosclerosis 2000; 149 (1): 163-8
46. Morris MS, Jacques PF, Selhub J, et al. Total homocysteine and estrogen status indicators in the third national health and nutrition examination survey. Am J Epidemiol 2000; 152 (2): 140-8
47. Berger PB, Herrmann RR, Dumensic DA. The effect of estrogen replacement therapy on total homocysteine in healthy postmenopausal women. Mayo Clin Proc 2000; 75 (1): 18-23

48. Green TJ, Houghton LA, Donovan U, et al. Oral contraceptives did not affect biochemical folate indexes and homocysteine concentrations in adolescent females. J Am Diet Assoc 1998; 98: 49-55
49. Anker G, Lonning PE, Ueland PM, et al. Plasma concentration of the atherogenic amino acid homocysteine in post-menopausal women with breast cancer treated with tamoxifen. Int J Cancer 1995; 60 (3): 365-8
50. Walsh BW, Paul S, Wild RA, et al. The effects of hormone replacement therapy and raloxifene on C-reactive protein and homocysteine in healthy postmenopausal women: a randomized controlled trial. J Clin Endocrinol Meta 2000; 85 (1): 214-8
51. Zmunda JM, Bausserman LL, Maceroni D, et al. The effect of supraphysiologic doses of testosterone on fasting total homocysteine concentration in normal men. Atherosclerosis 1997; 130: 199-202
52. Myllykangas-Luosujarvi R, Aho K, Kautiainen H, et al. Cardiovascular mortality in women with rheumatoid arthritis. J Rheumatol 1995; 22 (6): 1007-8
53. Wallberg-Jonsson S, Ohman ML, Dahlqvist SR. Cardiovascular morbidity and mortality in patients with sero positive rheumatoid arthritis in northern Sweden. J Rheumatol 1997; 24 (3): 445-51
54. Roubenoff R, Dellaripa P, Nadeau MR, et al. Abnormal homocysteine metabolism in rheumatoid arthritis. Arthritis Rheum 1997; 40 (4): 718-22
55. Haagsma CJ, Blom HJ, van Riel PLCM, et al. Influence of suphsalazine, methotrexate and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 1999; 58 (2): 79-84
56. Morgan SL, Baggott JE, Lee JY, et al. Folic acid supplementation prevents deficient blood folate concentration and hyper homocystenemia during long term, low dose methotrexate therapy for rheumatoid arthritis: implications for cardiovascular disease prevention. J Rheumatol 1998; 25 (3): 441-6
57. Ono H, Sakamoto A, Eguchi T, et al. Plasma total homocysteine concentrations in epileptic patients taking anti convulsants. Metabolism 1997; 46 (8): 959-62
58. Schwaninger M, Ringleb P, Winter R, et al. Elevated plasma concentrations of homocysteine in antiepileptic drug treatment. Epilepsia 1999; 40 (3): 345-50
59. Ueda K. Mechanism for reduction of folate by antiepileptic drugs during prolonged therapy. J Neurol Sci 1997; 145 (1): 109-12
60. Shorah CJ, Devitt H, Lucock M, et al. The responsiveness of plasma homocysteine to small increases in dietary folic acid: a primary care study. Eur J Clin Nutr 1998; 52: 407-11
61. Ubbink JB. Vitamin status and hyperhomocystenemia in a healthy population. In: Graham I, Refsum H, Rosenberg I, et al., editors. Homocysteine metabolism: from basic science to clinical medicine. Boston (MA): Kluwer Academic Publishers, 1994
62. Franken DG, Boers GH, Blom HJ, et al. Treatment of mild hyperhomocystenemia in vascular disease patients. Arterioscler Thromb 1994; 14: 465-70
63. Barber GW, Spaeth GL. Pyridoxine therapy in homocystinuria [letter]. Lancet 1967; I: 337
64. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosyl-methionine of the remethylation and transsulphuration of homocysteine. Am J Clin Nutr 1992; 55: 131-8
65. Lipsom MH, Kraus J, Rosenberg LD. Affinity of cystathionine beta-synthase for pyridoxal 5-phosphate in cultured cells: a mechanism for pyridoxine responsive homocystinuria. J Clin Invest 1980; 66: 188-93
66. Van den Berg M, Boers GH, Franken DG, et al. Hyperhomocysteinemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. Eur J Clin Invest 1995; 25: 176-81
67. Brattstrom L, Israesson B, Norrving B, et al. Impaired homocysteine metabolism in early onset cerebral and peripheral occlusive arterial disease: effects of pyridoxine and folic acid treatment. Arthrosclerosis 1990; 81: 51-60
68. Ubbink JB, Vermaak WJ, van der Merwe A, et al. Vitamin requirements for the treatment hyperhomocysteinemia in humans. J Nutr 1994; 124: 1927-33
69. Boers GHJ, van den Berg M, Franken DG. Treatment of mild hyperhomocysteinemia. In: Graham I, Refsum H, Rosenberg I, et al., editors. Homocysteine metabolism: from basic science to clinical medicine. Boston (MA): Kluwer Academic Publishers, 1994
70. van Gulener C, Janssen MJ, deMeer K, et al. Effect of folic acid and betaine on fasting and post methionine-loading plasma homocysteine and methionine concentration in chronic hemodialysis patients. J Intern Med 1999; 245 (2): 175-83
71. Woo KS, Chook P, Lolin Y, et al. Folic acid improves arterial endothelial function in adults with hyperhomocystenemia. J Am Coll Cardiol 1999; 34 (7): 2002-6
72. Chao CL, Chien KL, Lee YT. Effect of short term vitamin (folic Acid, vitamin B6 and B12) administration on endothelial dysfunction induced by post-methionine load hyperhomocysteinemia. Am J Cardiol 1999; 84 (11): 1359-61
73. Constans J, Blann AD, Resplandy F, et al. Three months supplementation of hyperhomocysteinemic patients with folic acid and vitamin B6 improves biological markers of endothelial dysfunction. Br J Haematol 1999; 107 (4): 776-8
74. Nappo F, De Rosa N, Marfella R, et al. Impairment of endothelial functions by acute hyperhomocysteinemia and reversal by antioxidant vitamins. JAMA 1999; 281 (22): 2113-8
75. Vermeulen EG, Stehouwer CD, Twisk JW, et al. Effect of homocysteine-lowering treatment with folic acid plus vitamin B6 on progression of subclinical atherosclerosis: a randomized, placebo-controlled trial. Lancet 2000; 355 (9203): 517-22
76. Van Guldener C, Janssen MJFM, Lambert J, et al. No change in impaired endothelial function after longterm folic acid therapy of hyperhomocysteinemia in haemodialysis patients. Nephro Dial Transplant 1998; 13: 106-12
77. Undas A, Domagala TB, Jankowski M, et al. Treatment of hyperhomocysteinemia with folic acid and vitamins B12 and B6 attenuates thrombin generation. Thromb Res 1999; 95: 281-8

Correspondence and offprints: Dr *Vivian Fonseca*, Tulane University School of Medicine, 1430 Tulane Avenue SL-53, New Orleans, LA 70112-2699, USA.
E-mail: vfonseca@tulane.edu