Review

Homocysteine and vascular disease

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For more than 20 years, moderately raised concentrations of total homocysteine (tHcy) have been associated with an increased risk of atherothrombotic vascular events but only recently has evidence mounted to suggest that the association may be causal. The association is independent of other factors, it is fairly consistent across many studies, it is strong and dose-related, and it is biologically plausible. However, the evidence needs to be strengthened by a systematic review of all comparable studies and the demonstration, in randomised trials, that lowering tHcy is followed by a significant reduction in atherothrombotic vascular disease. In addition, the measurement of tHcy needs to be standardised. If these can be achieved then tHcy measurement will become another useful marker of vascular risk, multivitamin therapy will be another therapeutic option for people at risk of atherothrombotic vascular disease, and fortification of food with folic acid will rise high on the political and public health agenda.

Only about two-thirds of all episodes of symptomatic atherothrombotic vascular disease in developed countries can be attributed to established genetic and environmental vascular risk factors.[1] An additional causal vascular risk factor may be raised plasma levels of homocysteine (hyperhomocysteinaemia). Although 30 years have elapsed since hyperhomocysteinaemia (and homocystinuria) were first associated with an increased risk of atherothrombotic vascular disease,[2] it is only recently that sufficient evidence has mounted to suggest that the association is independent and dose-related, and it remains to be established whether it is causal and modifiable.

Homocysteine in blood

Homocysteine is a sulphydryl-containing aminoacid derived from the metabolic demethylation of dietary methionine, which is abundant in animal protein. It is present in plasma in four forms: about 1% circulates as the free thiol; 70–80% is disulphide-bound to plasma proteins, chiefly albumin; and the remaining 20–30% combines with itself to form the dimer homocysteine or with other thiols, including cysteine, with which it forms the homocysteine-cysteine mixed disulphide.[3] The term "total plasma (or serum) homocysteine" (tHcy) refers to the combined pool of all four forms of homocysteine.

Definition of hyperhomocysteinaemia

An abnormal tHcy is defined by an arbitrary cut-off (eg, 95th percentile) in the distribution of concentrations found in the "normal population", in much the same way as hypertension and hypercholesterolaemia have been defined. For example, after methionine-loading, hyperhomocysteinaemia is defined as a tHcy of more than 2 SD above the mean.[4] Among fasting individuals, "normal" tHcy commonly ranges from 5 to 15 µmol/L,[5,6] and higher fasting values are classified arbitrarily as moderate (16–30), intermediate (31–100), and severe (>100 µmol/L) hyperhomocysteinaemia.

COLOUR
FIG TC

Homocysteine metabolism and possible mechanism of atherothrombotic disease

Homocysteine metabolism

Homocysteine is metabolised by remethylation or trans-sulphuration (figure).

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Panel 1: **Factors increasing plasma homocysteine levels**

Genetic defects in homocysteine metabolism
CβS
MTHFR
Methionine synthase

Nutritional deficiencies in vitamin cofactors
Folate
Vitamin B_{12} (cobalamin)
Vitamin B_6 (pyridoxine)

Diseases
Pernicious anaemia
Renal impairment
Hypothyroidism
Malignancy: acute lymphoblastic leukaemia; carcinoma of the breast, ovary and pancreas
Severe psoriasis

Medications/toxins
Folate antagonists (methotrexate, phenytoin, carbamazepine)
Vitamin B_6 antagonists (theophylline, azarabine, oestrogen-containing oral contraceptives, cigarette smoking)

Age/sex
Increasing age
Male sex
Menopause

Remethylation

Under conditions of low protein intake, homocysteine is metabolised primarily via one of two methionine-conserving remethylation pathways.[7] In the liver, a substantial proportion of homocysteine is remethylated by betaine-homocysteine methyltransferase with betaine as the methyl donor. In most other tissues, the remethylation is catalysed by methionine synthase with N^5-methyltetrahydrofolate as the donor. The formation of this methyl donor depends on the presence of N^5,N^{10}-methylenetetrahydrofolate (derived from dietary folate) and the enzyme N^5,N^{10}-methylenetetrahydrofolate reductase (MTHFR). Vitamin B_{12} (cobalamin) is an essential cofactor for methionine synthase.[7]

Trans-sulphuration

When the remethylation pathway is saturated, or when cysteine is required, homocysteine is converted to cystathionine (and then cysteine) by cystathionine β-synthase (CβS).[7] Vitamin B_6 (pyridoxine) is an essential cofactor. Cysteine may be metabolised further to sulphate and excreted in the urine.[7]

Factors influencing homocysteine metabolism (panel 1)

Genetic defects

The most common of the genetic causes of severe hyperhomocysteinaemia and classic homocystinuria (congenital homocystinuria) is homozygous deficiency of CβS. It occurs in only 1 in 100 000 live births and results in an increase of up to 40-fold in fasting tHcy. It is inherited as an autosomal recessive trait. The clinical features include dislocation of the lens and other ocular complications, intellectual handicap in perhaps 50%, skeletal deformities, premature atherosclerosis, and premature vascular (atherothrombotic) events. A vascular event occurs before the age of 30 years in about half of untreated homozygotes.[8] The heterozygous form (about 1 in 150 population) is often associated with normal basal tHcy, and it remains uncertain whether heterozygosity is associated with additional risk of vascular events. At least 60 mutations of the CβS gene have been described. I278T and G307S appear to be the most common though the prevalence of these mutations varies greatly between countries.[9] Even rarer causes of severe hyperhomocysteinaemia are homozygous deficiency of MTHFR, deficiency of methionine synthase, and impaired activity of methionine synthase due to genetic disorders of vitamin B_{12} metabolism.[10]

The most common enzyme defect associated with moderately raised tHcy is a point mutation (C-to-T substitution at nucleotide 677) in the coding region of the gene for MTHFR, which is associated with a thermolabile MTHFR variant that has about half-normal activity.[11] 10–13% of white populations are homozygous for this mutation (*TT* genotype),[12] and, in the presence of a suboptimal folate intake, will have a moderately raised (by about 50%) tHcy.[13]

Nutritional deficiencies

Because blood levels of folate, vitamin B_{12}, and, to a lesser extent, vitamin B_6 are related inversely to tHcy,[14] anyone with a nutritional deficiency that leads to low blood concentrations of folate, vitamin B_{12}, or vitamin B_6 is at increased risk of hyperhomocysteinaemia.[15] Indeed, it has been suggested that about two-thirds of hyperhomocysteinaemia is due to inadequate blood levels of one or more of these vitamin cofactors.[14]

Other causes

Renal impairment commonly causes hyperhomocysteinaemia. Fasting tHcy rises as serum creatinine rises, not because of impaired urinary excretion (which is a very minor route for direct homocysteine clearance) but because of impaired metabolism of homocysteine by the kidney, the major route by which homocysteine is cleared from plasma.[16] Total homocysteine levels are considerably higher in patients with chronic renal disease than the moderately raised concentrations commonly found in patients with atherothrombotic vascular disease, and this may contribute to the high incidence of vascular complications in patients with chronic renal failure.

Plasma homocysteine concentrations can be increased by various drugs and diseases that interfere with folate, vitamin B_6, and B_{12} metabolism, and an abnormal homocysteine concentration may therefore be used as a diagnostic aid for some of these conditions.[17]

Measurement of plasma homocysteine

The most robust measure is tHcy, which is assumed to represent biologically active homocysteine, directly or indirectly.[3] The most widely used method is high-performance liquid chromatography (HPLC),[5] but a simple, reliable, rapid, and inexpensive immunoassay may replace it.[18] The measurement can be done fasting or non-fasting, and before and after oral methionine-loading. We recommend that tHcy be measured after fasting for at least 12 h, to avoid the variable rise in tHcy that may occur after a meal.[19]

Methionine-loading stresses the homocysteine metabolic pathways and is more sensitive than fasting levels are to mild disturbances in the trans-sulphuration pathway such as caused by deficiency of vitamin B_6 or partial CβS deficiency. The procedure involves measuring baseline tHcy, ingestion of a standard oral dose of methionine (0·1 g/kg body weight or 3·8 or 4·0 g/m^2 body surface area) and

Panel 2: **Homocysteine and cardiovascular risk, prospective cohort and nested case-control studies***

Study	Sex	Cases/control	Major endpoints	tHcy (µmol/L)		RR (95% CI)†
				Cases	Controls	
Physicians' Health Study,[30] USA	M	271/271	Fatal/nonfatal MI and CHD death	11·1	10·5	3·4 (1·3–8·8)
BUPA,[31] UK	M	229/1126	Fatal CHD	⩾15·2	<10·3	2·9 (2·04–4·1)
Tromso,[32] Norway	M/F	123/492	Fatal/nonfatal CHD	12·7	11·3	1·32 (1·05–1·65)
British Regional Heart Study[33]	M	107/118	Fatal/nonfatal stroke	13·7	11·9	2·8 (1·3–5·9)
Nygard,[34] Belgium	M/F	64 cases only	Death	⩾20	⩽9·0	4·5 (1·22–16·6)
Physicians' Health Study,[35] USA	M	333/333	Fatal/nonfatal MI and CHD death	–	–	1·7 (0·9–3·3)
Physicians' Health Study,[36] USA	M	149/149	New angina, CABG	10·9	10·4	1·0 (0·4–2·4)
Physicians' Health Study,[37] USA	M	109/427	Ischaemic stroke	11·4	10·6	1·2 (0·7–2·0)
MRFIT,[38] USA	M	93/186	Nonfatal MI	12·6	13·1	0·82 (0·55–54)
	M	147/286	CHD death	12·8	12·7	. .
North Karelia Project,[39] Finland	M/F	265/269	Fatal/nonfatal MI, stroke	M 9·9 F 9·6	M 9·8 F 9·3	M 1·05 (0·56–1·95) F 1·22 (0·6–2·78)
ARIC,[40] USA	M/F	232/537	Fatal/nonfatal MI	8·9	8·5	1·28 (0·5–3·2)

CABG=coronary artery bypass graft; CAD=coronary artery disease; CHD=coronary heart disease.
*For further details of selection, age, and length of follow-up see original papers.
†RR for tHcy >95th percentile of control population (ref 30, 36, 39); for highest versus lowest quartile of tHcy (refs 31, 33, 36, 39); for per 4 µmol/L increment in tHcy (ref 32); for highest versus lower four quintiles of tHcy (ref 37); or for highest versus lowest quintile of tHcy (ref 40).

a repeat measure of tHcy after a fixed time of between 2 and 8 h after ingestion. Methionine-loading may have a particular role in discriminating between defects involving the trans-sulphuration and remethylation pathways,[6] and may also identify patients who have impaired homocysteine metabolism despite a normal fasting tHcy, and who may therefore be at increased risk of vascular disease.[20] However, this method is inconvenient for clinician and patient, methionine has an unpleasant taste, and the reference values for post-load results are uncertain.

Blood specimens should ideally be centrifuged immediately because delay leads to a time and temperature dependent release of homocysteine from blood cells.[6] If immediate centrifugation is not possible, the specimen should be placed directly on ice until the plasma is separated.

The day-to-day variation in fasting tHcy among healthy individuals is small (coefficient of variation 7%), so a single measurement is reasonable.[21] Men have a tHcy that is about 1 µmol/L higher than that of women, and concentrations tend to increase slightly with age.[22] Two studies have reported tHcy to be up to 40% lower during the first 24 hours of an acute occlusive vascular event than on repeat measures at least 72 h later,[23,24] but tHcy was not measured before the events so it remains uncertain whether tHcy really is depressed in the acute phase or whether the event triggers a subsequent increase in tHcy due to a series of physiological changes or dietary deficiency.

Assay standardisation is rudimentary compared with that for measurements such as cholesterol. Most analyses of tHcy using HPLC have similar distributions and reference ranges,[25] but imprecise estimates, related in part to instability of the standard commonly used for calibration.[26] The European Research Network on Inherited Disorders of Metabolism provides an external quality assessment of assays used to monitor the high tHcy found with inborn errors of metabolism, and has helped raise general awareness of the need for standardisation.

Prevalence of hyperhomocysteinaemia

The prevalence of hyperhomocysteinaemia has been estimated to be 5% in the general population, and 13–47% among patients with symptomatic atherosclerotic vascular disease.[27,28] However, these estimates are based on a cut-off above the 90th or 95th percentile of the distribution of tHcy in the general population. We think it more appropriate to define hyperhomocysteinaemia as a plasma level of tHcy that correlates with an increased risk of atherosclerotic vascular events. Unfortunately, there is no definite threshold that correlates with a sudden increase in the risk of vascular events—indeed, the relation between tHcy and risk appears to be linear (or log-linear), in much the same way that increasing blood pressure and cholesterol are related to vascular disease. Estimates of the prevalence of hyperhomocysteinaemia are further confounded by uncertainty surrounding the validity of measures of tHcy. There are many different methods of measuring tHcy but no standards against which different laboratories and methods can be compared.

Homocysteine and atherothrombotic vascular disease

Atherothrombotic vascular events were linked to raised tHcy in patients with homocystinuria in 1969, but it was not until 1976 that a controlled study showed a clear association between moderately raised tHcy and atherosclerotic disease.[29] Since then, a possible association between tHcy (or the common thermolabile MTHFR gene mutation associated with hyperhomocysteinaemia) and atherothrombotic vascular disease has been examined in more than 12 000 patients in more than 100 cross-sectional, case-control, and prospective cohort studies. A systematic review of data from these observational studies is complicated by variations in study design, type, and number of patients and controls; in the measurement of tHcy, in definitions of hyperhomocysteinaemia and definitions and measurement of other vascular risk factors and possible confounding factors, in methods of follow-up, types and definitions of vascular outcome events (and surrogate outcome measures); and in statistical analyses. Despite these reservations, we have reviewed and interpreted the epidemiological data as follows.

Prospective cohort studies

The strongest epidemiological evidence for an association between hyperhomocysteinaemia and vascular risk comes from large prospective observational cohort studies. The 11 studies (panel 2) are not consistent. The unequivocally

Panel 3: **Homocysteine and cardiovascular risk, cross-sectional and retrospective case-control studies involving at least 150 cases published since meta-analysis by Boushey et al[41] in 1995***

Study	Sex	Cases/control	tHcy (μmol/L) Cases	tHcy (μmol/L) Controls	Main results†
Graham,[20] Europe	M/F	750/800	11·3	9·7	RR=2·2 (1·6–2·9)
Lingren,[23] Sweden	M/F	162/60	13·4	13·8	NS
Hopkins,[42]USA	M/F	162/155	M 13·7	M 11·3	p=0·0001
			F 12·6	F 8·9	p=0·0001
					RR=8·1 (3·2–20·4)
Dalery,[43] Canada	M/F	150/584	M 11·7	M 9·7	p<0·001
			F 12·0	F 7·6	p<0·01
Robinson,[44] USA	M/F	304/231	M 13·9	M11·2	p<0·01
			F 15·3	F 10·1	p<0·01
Malinow,[45] France and Ireland	M	420/521	Ireland 15·5	Ireland 14·7	OR=1·84 (0·8–4·5)
			France 16·7	France 12·9	OR=4·27 (2·0–9·3)
Markus,[46] UK	M/F	160/75	1·32‡	1·27‡	p=0·09

*For further details of selection, age, and length of follow-up see original papers.
†Given as p values for tHcy in cases versus controls or as RR/OR for highest versus lower for quintiles of tHcy (ref 20); for highest versus lowest quintile (ref 46); or for 10 μmol/L increment (ref 42).
‡Log tHcy.

positive studies—with relative risks (RR) whose lower 95% confidence interval is above 1—are the Physicians' Health Study, the British United Provident Association study, the Tromso study, the British Regional Heart Study, and the study of Nygard et al.[34] A strong graded relation has been reported between increasing tHcy and overall mortality in individuals with angiographically demonstrated coronary heart disease.[34] However, other prospective studies, including further reports from the Physicians' Health Study, have not found a significant association between hyperhomocysteinaemia and myocardial infarction or stroke (panel 2).

Cross-sectional and retrospective case-control studies

Retrospective and cross-sectional designs are methodologically less rigorous than cohort designs but most of them strongly support an association between tHcy and vascular disease risk. In 1995, Boushey et al[41] reported a meta-analysis of 27 observational studies (23 cross-sectional or retrospective case-control and four nested case-control studies based on prospective cohorts), including about 4000 patients. A raised tHcy (usually defined as above the 90th or 95th percentile of controls) was associated with an increased risk of fatal and nonfatal atherosclerotic vascular disease in the coronary (odds ratio 1·7; 95% CI 1·5–1·9), cerebral (OR 2·5 [2·0–3·0]), and peripheral (OR 6·8 [2·9–15·8]) circulations. The magnitude of risk was similar to that for other risk factors, such as hypercholesterolaemia and smoking, and it was estimated that about 10% of coronary heart disease in the general population might be attributable to homocysteine.[41] Boushey et al[41] also estimated, from an analysis that assumed a graded, linear relation between homocysteine levels and vascular risk, that a 5 μmol/L increase in tHcy was associated with an increase in vascular risk of about one-third, which is of similar magnitude to an increase in plasma cholesterol of 0·5 mmol/L.

Since that 1995 meta-analysis, there have been over 40 more case-control studies, most of them supporting the conclusions of Boushey et al.[41] Panel 3 lists the studies on 150 or more cases.[20,23,42–46] The large European Collaborative Study concluded that tHcy was an independent risk factor for atherosclerotic disease, and calculated that a 5 μmol/L increment in fasting basal tHcy was associatd with RR of atherosclerotic vascular disease of 1·35 (1·1–1·6) in men and 1·42 (0·99–2·55) in women.[20]

Homocysteine and extent of atherosclerotic vascular disease

Published cross-sectional and case-control studies demonstrate a clear association between tHcy and the anatomical extent of carotid,[47–48] coronary,[49–50] aortic,[51] and peripheral[52] vascular disease but these are only surrogate measures of vascular events.

Genetic defects

Frosst et al proposed the C677T polymorphism in the MTHFR gene as a candidate for vascular disease risk,[12] but most individual studies and a recent meta-analysis of more than 6000 genotyped patients and controls[53] have failed to confirm this. We cannot explain why the *TT* genotype in the MTHFR gene, which is a strong predictor of raised tHcy in the general population, is not asociated with an increased risk of vascular disease (if hyperhomocysteinaemia really is associated with vascular disease). One explanation may be that many of the studies involved large numbers of individuals with an adequate folate intake (and hence unremarkable tHcy). An alternative explanation could be that the *TT* genotype causes a redistribution of the different folate metabolites, leading to a higher availability of one-carbon moieties for thymidine synthesis, which could have a beneficial effect on the cardiovascular system.[54]

Homocysteine and venous thrombosis

Most retrospective case-control and cross-sectional studies have demonstrated a positive association between raised tHcy and an increased risk of initial and recurrent venous thrombosis, and this has been confirmed by a meta-analysis (RR=2·95 [2·08–4·17]) in patients with fasting hyperhomocysteinaemia.[55] Supporting evidence comes from the Physicians' Health Study cohort[56] (RR=3·38 [1·57–7·25] in patients with a tHcy above the 95th percentile of controls). However, an association was not found between tHcy and risk of venous thrombosis in a prospectively studied cohort of patients with systemic lupus erythematosus.[57]

Interpretation

There is a large body of epidemiological evidence that links increasing tHcy with an increasing risk of atherothrombotic vascular disease. There is not complete consistency but

there hardly ever is, even for well-established relationships, usually because of differences in study design, statistical power, and chance. Whilst it is possible that the abundance of published studies that show a positive association could reflect publication bias it is just as likely that the negative studies are falsely negative because of a lack of methodological or statistical power or random error. A systematic review of studies with the same types of patients and controls and the same methods and outcome events could provide a more accurate estimate of the true association between tHcy and vascular risk.

Assuming that a strong, dose-dependent, and positive association does exist, there is reasonable evidence, from regression analyses, that the association is independent of other factors known to be associated with raised tHcy (eg, age, sex, smoking, low physical activity, blood pressure, and cholesterol).[58] Furthermore, homozygotes for the three distinct autosomal-recessive inborn errors of homocysteine metabolism (CβS deficiency, MTHFR deficiency, and the cobalamin metabolic defects that impair methionine synthase activity) have tHcy concentrations that are 10–50 times higher than those in the general population and a very high risk of premature atherothrombotic vascular disease. This suggests that the only biochemical change these metabolic errors have in common (ie, a high circulating tHcy or homocysteine metabolite concentration) is the cause of the premature atherothrombotic vascular disease that is shared by all three disorders.

However, the conclusion that tHcy is an independent risk factor for atherothrombotic vascular disease should be tempered by the variable findings of prospective cohort studies, and the failure to demonstrate a clear association between genetic markers of raised tHcy (notably, the thermolabile MTHFR genotype), and vascular disease despite a significant relation between genotype and tHcy. Furthermore, the more positive results of retrospective studies may reflect a consistent bias resulting from homocysteine levels measured after the acute vascular event, which may be higher than levels measured beforehand. Finally, it is also possible that tHcy is simply a marker of another causal risk factor such as folate status or some other, unknown factor which could explain the higher rates of CHD among people with lower intakes of folate and vitamin B_6 (eg, folate-depleted diets may be prothrombotic).

Biological plausibilty of epidemiological association

The mechanism by which homocysteine might cause vascular damage is unclear. Experimental evidence suggests that homocysteine promotes atherogenesis by facilitating oxidative arterial injury, damaging the vascular matrix, and augmenting the proliferation of vascular smooth muscle. It may promote thromboembolic disease by causing oxidative injury to the endothelium, altering the coagulant properties of the blood, and impairing endothelium-dependent vasomotor regulation (figure).[59] However, most in-vitro and volunteer studies have been done with homocysteine concentrations at least 10 times greater than those seen in patients with moderate hyperhomocysteinaemia, and should therefore be interpreted with caution. An atherogenic and/or prothrombotic effect of homocysteine remains unproven.

Therapies which lower plasma homocysteine

Although specific therapy for hyperhomocysteinaemia is best tailored to the underlying cause, more than 90% of patients respond to multivitamin treatment within 2–6 weeks, irrespective of the cause.

Folic acid (pterolymonoglutamic acid) is the synthetic, heat-stable chemical that is about twice as bioavailable as folate, the generic term for compounds that occur naturally in food.[60] The major sources of folate in the diet are fortified breakfast cereals, fortified bread, and fruit and vegetables. Folic acid therapy alone or combined with vitamin B_6 and vitamin B_{12}, reduces tHcy even in people who are not frankly vitamin deficient.[61,62] Folic acid is the single most effective therapy for hyperhomocysteinaemia. Patients with renal impairment require much higher doses. A meta-analysis of data from 1114 individual participants in 12 randomised controlled trials of the effects of folic-acid-based supplements on basal tHcy found that the proportional and absolute reductions in tHcy produced by folic acid were greater at higher pretreatment tHcy and at lower pretreatment blood folate levels.[62] After standardisation to pretreatment baseline levels of 12 µmol/L for homocysteine and 12 nmol/L for folate (approximate average levels for western populations), dietary folic acid (0·5–5 mg) was asociated with a reduction in basal tHcy of about 25% (95% CI 23–28%); vitamin B_{12} (mean 0·5 mg daily) was associated with an additional reduction in basal tHcy of 7% (3–10%); vitamin B_6 (mean 16·5 mg daily) did not have a signifcant additional effect.[62]

The minimum effective daily dose of folic acid for achieving maximal homocysteine-lowering efficacy is about 400 µg.[13,28,41] Higher daily doses are no more effective[62] (except in renal failure), but whether doses lower than 400 µg are effective has not been adequately explored. Because the response to homocysteine-lowering therapy is not uniform, and is dependent on factors such as genotype for enzymes involved in the metabolism of homocysteine, vitamin status, and nutritional needs,[13] multivitamin doses required for the treatment of hyperhomocysteinaemia may vary according to individual patient requirements.

A potential hazard of folic acid therapy is progressive neurological damage (subacute combined degeneration of the spinal cord) in people with subclinical vitamin B_{12} deficiency in whom folic acid therapy may mask the development of the haematological manifestations of the B_{12} deficiency. This can be avoided by either excluding B_{12}

Panel 4: **Ongoing large randomised controlled clinical trials of homocysteine-lowering therapy in vascular disease**

Bergen Vitamin Study, University of Bergen, Norway
Cambridge Heart Antioxidant Study (CHAOS-2); University of Cambridge, UK
Heart Outcomes Prevention Evaluation (HOPE-2) Study; McMaster University, Canada
Norwegian Study of Homocysteine Lowering with B-vitamins in Myocardial Infarction (NORVIT); University of Tromso, Norway
Prevention with A Combined Inhibitor and Folate in Coronary Heart Disease (PACIFIC) Study; University of Sydney, Australia
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH); Clinical Trial Service Unit, Oxford, UK
Vitamins in Stroke Prevention (VISP) Trial; Bowman Gray School of Medicine, USA
VITAmins TO, Prevent Stroke (VITATOPS) Study; Stroke Unit, Royal Perth Hospital, Western Australia
Women's Antioxidant and Cardiovascular Disease Study (WACS); Harvard Medical School, USA

deficiency before starting folic acid or by supplementing folic acid therapy with vitamin B_{12}. At least 400 µg per day of vitamin B_{12} is suggested as a supplement because the recommended daily intake of this vitamin is about 2 µg per day but only 1–3% of oral vitamin B_{12} is absorbed by simple diffusion.[63] The major potential hazard of vitamin B_6 is a sensory peripheral neuropathy with use over months to years at doses of vitamin B_6 usually at least 400 mg daily. However, most doses for the treatment of moderate hyperhomocysteinaemia are only 10–50 mg per day.

In patients with severe hyperhomocysteinaemia due to CβS deficiency effective homocysteine-lowering therapy does reduce the risk of cardiovascular disease during long-term follow-up.[64] However, although the combination of folic acid 2·5 mg, vitamin B_6 25 mg, and vitamin B_{12} 250 µg per day reduces the progression of atherosclerosis, as measured by carotid plaque area,[65] it remains to be confirmed that homocysteine-lowering therapy will prevent important atherosclerotic vascular events in patients with moderate hyperhomocysteinaemia. Several large randomised clinical trials are addressing this issue (panel 4).

Because folate deficiency among pregnant women is a risk factor for serious neural-tube birth defects, the US Public Health Service in 1992 recommended that women who could become pregnant should consume 400 µg of folic acid daily.[66] Since Jan 1, 1998 the US Food and Drug Administration has required enriched grains to be fortified with folic acid at a concentration that will provide the average woman with an extra 100 µg per day. It is not known how increasing recommendations for the use of folic acid supplements in the general population and the introduction of folate fortification of grains will affect intervention trials, but the fortification programme in the USA is likely to lower the homocysteine levels of any control group, biasing the study toward the null hypothesis and reducing the power of the study. However, the daily dose of folic acid required to maximally lower tHcy is about four times higher than the 100 µg per day obtained by fortification of grains. Malinow et al[28] recently demonstrated that although cereals providing 127 µg of folic acid daily increase plasma folate levels by 30%, tHcy was reduced by only a mean of 3·7%. In contrast, cereals providing in excess of 400 µg of folic acid daily reduced tHcy by a mean of at least 11%, suggesting that folic acid fortification at levels higher than that currently recommended may be warranted.

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