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DACH-LIGA Homocystein (German, Austrian and Swiss Homocysteine Society): Consensus Paper on the Rational Clinical Use of Homocysteine, Folic Acid and B-Vitamins in Cardiovascular and Thrombotic Diseases: Guidelines and Recommendations

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About half of all deaths are due to cardiovascular disease and its complications. The economic burden on society and the healthcare system from cardiovascular disability, complications, and treatments is huge and getting larger in the rapidly aging populations of developed countries. As conventional risk factors fail to account for part of the cases, homocysteine, a "new" risk factor, is being viewed with mounting interest.

Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine, an essential amino acid. Folic acid, vitamin B_{12}, and vitamin B_6 deficiencies and reduced enzyme activities inhibit the breakdown of homocysteine, thus increasing the intracellular homocysteine concentration. Numerous retrospective and prospective studies have consistently found an independent relationship between mild hyperhomocysteinemia and cardiovascular disease or all-cause mortality. Starting at a plasma homocysteine concentration of approximately 10 µmol/l, the risk increase follows a linear dose-response relationship with no specific threshold level. Hyperhomocysteinemia as an independent risk factor for cardiovascular disease is thought to be responsible for about 10% of total risk. Elevated plasma homocysteine levels (> 12 µmol/l; moderate hyperhomocysteinemia) are considered cytotoxic and are found in 5 to 10% of the general population and in up to 40% of patients with vascular disease. Additional risk factors (smoking, arterial hypertension, diabetes, and hyperlipidemia) may additively or, by interacting with homocysteine, synergistically (and hence over-proportionally) increase overall risk. Hyperhomocysteinemia is associated with alterations in vascular morphology, loss of endothelial anti-thrombotic function, and induction of a procoagulant environment. Most known forms of damage or injury are due to homocysteine-mediated oxidative stress. Especially when acting as direct or indirect antagonists of cofactors and enzyme activities, numerous agents, drugs, diseases, and lifestyle factors have an impact on homocysteine metabolism. Folic acid deficiency is considered the most common cause of hyperhomocysteinemia. An adequate intake of at least 400 µg of folate per day is difficult to maintain even with a balanced diet, and high-risk groups often find it impossible to meet these folate requirements. Based on the available evidence, there is an increasing call for the diagnosis and treatment of elevated homocysteine levels in high-risk individuals in general and patients with manifest vascular disease in particular. Subjects of both populations should first have a baseline homocysteine assay. Except where manifestations are already present, intervention, if any, should be guided by the severity of hyperhomocysteinemia. Consistent with other working parties and consensus groups, we recommend a target plasma homocysteine level of < 10 µmol/l. Based on various calculation models, reduction of elevated plasma homocysteine concentrations may theoretically prevent up to 25% of cardiovascular events. Supplementation is inexpensive, potentially effective, and devoid of adverse effects and, therefore, has an exceptionally favorable benefit/risk ratio. The results of ongoing randomized controlled intervention trials must be available before screening for, and treatment of, hyperhomocysteinemia can be recommended for the apparently healthy general population. Clin Chem Lab Med 2003; 41(10): 1392–1403

Key words: Homocysteine; Hyperhomocysteinemia; Vitamin B_{12}; Folate; Therapy.

Abbreviations: 5-methyl-THF, 5-methyltetrahydrofolate; CAD, coronary artery disease; CBS, cystathionine-β-synthase; DFE, dietary folate equivalents; FNB, United States Food and Nutrition Board; MTHFR, 5,10-methylenetetrahydrofolate reductase; PAOD, peripheral arterial occlusive disease; RDA, recommended daily amounts; SAM, S-adenosylmethionine; UL, upper intake level.

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Introduction

Each year about 4 million Europeans die from cardiovascular disease and its complications (coronary artery disease (CAD), peripheral arterial occlusive disease (PAOD), myocardial infarction, stroke, venous thrombosis). In the three DACH countries (Germany, Austria, Switzerland), there were 443,498 cardiovascular deaths in 2001 (www.statistik.at, www.destatis.de, www.statistik.admin.ch), accounting for 46% of all deaths there (1, 2). The economic burden on society and the healthcare system from cardiovascular disability, complications, and treatments is huge and getting larger in the rapidly aging populations of developed countries (2–5). Atherosclerosis is today considered a chronic condition that progresses in bouts rather than as a continuous process (6). Atherosclerosis is often detectable at a young age and therefore amenable to early, efficient prophylaxis (7, 8). There is therefore an increasing call for starting risk factor identification at the age of 20 years, and absolute individual risk should be known when a person turns 40 years (9, 10).

Hyperhomocysteinemia as an independent risk factor for cardiovascular disease is thought to be responsible for about 10% of total risk (11, 12). Based on various calculation models, reduction of elevated plasma homocysteine concentrations may prevent up to 25% of cardiovascular events (13–15). Based on the available evidence, there is an increasing call for the diagnosis and treatment of elevated homocysteine levels in high-risk populations (5, 7, 12, 16–19). The results of ongoing randomized controlled intervention trials must be available before screening for, and treatment of, hyperhomocysteinemia can be recommended for the apparently healthy general population (20). Apart from its significance as an independent risk factor of additional prognostic value, homocysteine is a sensitive diagnostic indicator of folate, vitamin B_{12}, and vitamin B_6 deficiencies (21–23). The determination of the plasma homocysteine concentration is also useful for documenting response to vitamin supplementation.

The purpose of this consensus paper is to provide orientation on how to handle the risk factor homocysteine in terms of its diagnostic and clinical roles in atherothrombotic conditions and the need for therapeutic intervention.

Metabolism and Pathobiochemistry

Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine, an essential amino acid (Figure 1). "Activated" S-adenosylmethionine (SAM) is the most important methyl donor in numerous biological reactions (DNA, proteins, neurotransmitters, hormones, phospholipids) (24). Acquiring a methyl group from 5-methyltetrahydrofolate (5-methyl-THF), homocysteine is remethylated to methionine. This reaction is catalyzed by the enzyme methionine synthase, and vitamin B_{12} is required as a cofactor. Alternatively, homocysteine can, by condensation with serine and *via* cystathionine, be irreversibly broken down to cysteine and glutathione (transsulfuration). The activities of both enzymes involved in this metabolic pathway, *i.e.*, cystathionine-β-synthase (CBS) and γ-cystathionase, depend on the cofactor vitamin B_6.

In addition to their functions as cofactors for the enzymes involved in homocysteine metabolism, vitamins B_{12}, B_6, and folic acid have yet other important, independent properties (25–28). Folic acid and vitamin B_6 deficiencies are independent risk factors for cardiovascular disease. Apart from being involved in the development of hyperhomocysteinemia, folate de-

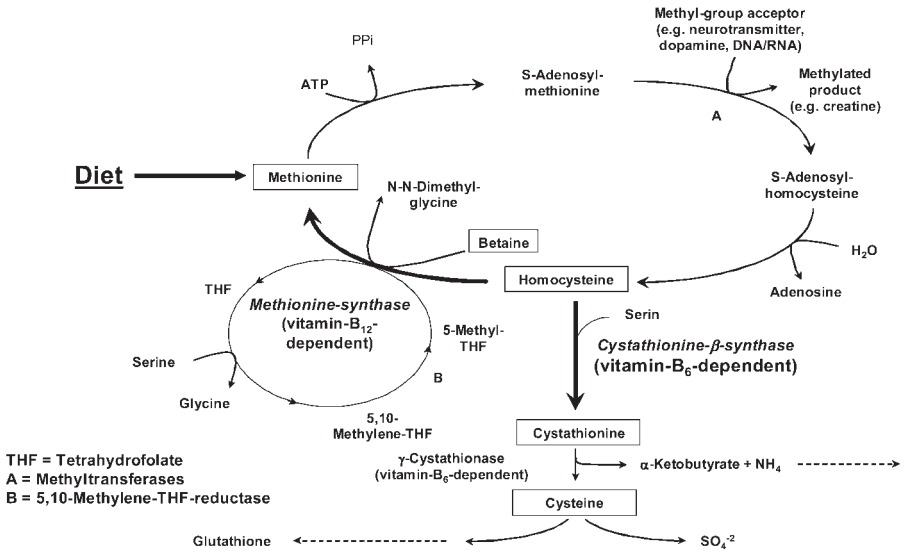

Figure 1 Homocysteine metabolism (THF = tetrahydrofolate, A = methyltransferases, B = 5,10-methylenetetrahydrofolate reductase).

ficiency is associated with hypomethylation, DNA damage (chromosome strand breaks), or impaired cell proliferation with an increased risk of malignant disease (29, 30). As vitamin B_{12} acts as a cofactor for methionine synthase and is involved in folate metabolism, vitamin B_{12} deficiency may, even with adequate folate intake, lead to reduced remethylation as well as to hypomethylation (31). This results in elevated plasma homocysteine levels and functional folate deficiency, despite (seemingly) adequate plasma folic acid concentrations (because folate is "trapped" as methyltetrahydrofolate).

Folic acid, vitamin B_{12}, and vitamin B_6 deficiencies and reduced enzyme activities inhibit the breakdown of homocysteine, thus increasing the concentration of intracellular homocysteine (32–34). Being cytotoxic, homocysteine is increasingly exported from the cell to become detectable in plasma.

Homocysteine is present in plasma (serum) in various forms in different proportions. The free, reduced form accounts for less than 2%, while most homocysteine in plasma is present in oxidized form bound to albumin or as the disulfide (35). Only minute amounts of homocysteine are found in the urine of healthy subjects. The term "homocystinuria" should therefore be reserved for inborn errors of metabolism characterized by extremely elevated plasma homocysteine levels and substantially increased excretion of homocysteine in the urine.

Homocysteine as a Risk Factor

Numerous retrospective and prospective studies have consistently found an independent relationship between mild hyperhomocysteinemia (fasting or after oral methionine loading) and cardiovascular disease or all-cause mortality (11, 14, 16, 32, 36–38). Starting at a plasma homocysteine concentration of approximately 10 µmol/l, an associated risk increase follows a linear dose-response relationship with no specific threshold level (11, 19, 22, 30, 39, 40). Practically all essential criteria for a causal association (41) between cardiovascular events and elevated homocysteine concentrations are considered met (11, 15). The importance of homocysteine as a risk factor is approximately equivalent to that of smoking or hyperlipidemia (11, 12); relative risk is at least 1.3 to 1.7 for a 5 µmol/l increase in plasma homocysteine (14, 32) and is further increased in pre-existing vascular disease. Meta-analyses have calculated that homocysteine is responsible for at least 10% of the total risk for atherothrombotic vascular disease (12, 13, 15).

Additional risk factors (smoking, arterial hypertension, diabetes, and hyperlipidemia) may additively or, by interacting with homocysteine, synergistically (and hence overproportionally) increase overall risk (12, 32, 42–44). Meta-analyses have calculated that a 3 to 5 µmol/l reduction in plasma homocysteine may reduce the incidence of venous thrombosis, stroke, and CAD mortality by up to 25% (14, 15).

Causes of Hyperhomocysteinemia

Age and gender

Plasma homocysteine increases with age, and younger men normally have higher levels than women the same age. In people around the age of 40 years, the gender difference is approximately 2 µmol/l and can be explained by the effect of estrogen in women because it disappears rapidly after menopause. The age-related increase in plasma homocysteine can be explained, at least in part, by the physiological decline in renal function with age. Plasma homocysteine levels show an essentially linear increase up to the age of 60–65 years but rise much faster thereafter, increasing by approximately 10% or 1 µmol/l per decade (16, 32).

Genetic factors

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) irreversibly reduces 5,10-methylene-THF to 5-methyl-THF. About 5 to 15% of the general population in Germany, Austria, and Switzerland are homozygous carriers of a thermolabile variant of MTHFR that is due to a point mutation at nucleotide position 677 (MTHFR 677C→T) (45). MTHFR activity is reduced by approximately 70% in affected individuals. Carriers of the mutation are therefore particularly sensitive to folate deficiency, experiencing an increase in plasma homocysteine by approximately 25% (or about 2.6 µmol/l) (33). Recent meta-analyses including sufficiently large numbers of cases have found an associated 16 to 23% risk increase for homozygotes, explained by the plasma homocysteine increase or folate deficiency (15, 46–48). Approximately 1% of the general population is heterozygous for mutations in the *CBS* gene. Carriers of this mutation show elevated homocysteine concentrations after oral methionine loading and also have an increased risk of vascular disease (19). Other mutations with a possible impact on homocysteine metabolism (methionine synthase (49), methionine synthase reductase (50), *etc.*) are very rare, and their clinical significance is all but unexplored.

Vitamin deficiency

Vitamin deficiency is by far the leading cause of hyperhomocysteinemia, and it may be due to inadequate intake, reduced absorption from the gastrointestinal tract, increased consumption, and (drug) interactions. Individuals who do not eat a balanced diet (*e.g.*, vegetarians), elderly people, pregnant women, patients with renal disease, malabsorption (inflammatory bowel disease) or malignant disease are at risk of clinically significant vitamin deficiency. In addition, alcohol abuse and use of certain drugs (see Table 1) may lead to vitamin deficiency. Folate deficiency is the most common vitamin deficiency in Europe, partly because of a lack of fresh fruits and vegetables. Good dietary sources of folates include green vegetables, cereals, fruits, yeast, and liver (with reservations). However, up to 90% of folates may be lost during processing of ce-

Table 1 Causes of plasma homocysteine (Hcy) changes.

Cause	Hcy	Mechanism
Drugs		
Theophylline	↑	Vitamin B_6 antagonist; inhibits pyridoxal kinase
Nitrous oxide (N_2O)	↑	Oxidation of cobalt, cobalamin and methionine synthase inactivation
Lipid-lowering drugs		
Fibrates	↑	PPARα activation? Renal function?
Niacin	↑	Vitamin B_6 antagonist; inhibits pyridoxal kinase
Colestipol/cholestyramine	↑	Impairment of folic acid and cobalamin absorption
Antifolates		
Methotrexate	↑	Inhibits dihydrofolate reductase, folic acid antagonist
Trimethoprim	↑	Inhibits dihydrofolate reductase
Hormones		
Postmenopausal HRT	↓	Estrogen effect
Oral contraceptives	↑ (?)	Interference with folic acid? (relevance still unclear)
Antiepileptic drugs	↑	Folic acid antagonism, enzyme modulation
Metformin	↑	Inhibition of vitamin B_{12} absorption, binding of Ca^{2+}
Omeprazole	↑	Impairment of vitamin B_{12} absorption
Mesna	↓	Disulfide exchange
Levodopa	↑	Levodopa is a substrate for SAM-dependent methylation
D-Penicillamine	↓	Disulfide exchange
N-Acetylcysteine	↓	Disulfide exchange
Antiestrogens		
Tamoxifen	↓	Partial estrogen antagonist, enzyme induction?
Raloxifene	↓	Enzyme induction?
Aminoglutethimide	↑	Enzyme induction?
Cyclosporin A	↑	Renal function?
Sulfasalazine	↑	Inhibits dihydrofolate reductase and folate absorption
Isoniazid	↑	Vitamin B_6 antagonist through complex formation
(Hyper)proliferative conditions		
Psoriasis	↑	Cell proliferation
Acute lymphocytic leukemia	↑	Cell proliferation
Rheumatoid arthritis	↑	Cell proliferation
Thyroid disorders		
Hypothyroidism	↑	Enzyme induction
Hyperthyroidism	↓	Enzyme induction
Renal impairment	↑↑	Impaired remethylation
Smoking	↑	Interference with vitamin B_6, B_{12}, and folate; redox
Coffee/caffeine	↑	Vitamin B_6 antagonist (caffeine), methyl group requirements ↑
Alcohol	↑	Interference with vitamin B_6, B_{12}, and folate; enzyme inhibition

HRT = hormone replacement treatment; PPARα = peroxisome proliferator-activated receptor-α; SAM = S-adenosylmethionine.

reals and other foods (51). Folates are also lost because folic acid is sensitive to heat, storage, and light. A number of professional associations recommend five servings of fruits and vegetables a day (600–700 g), but most people find it all but impossible to comply with this recommendation. An average daily intake of approximately 400 µg of dietary folate equivalents (DFE) would optimize all folate-dependent metabolic parameters (*e.g.*, homocysteine). However, the average daily dietary folate intake in Germany, Austria, and Switzerland is currently clearly below 300 µg (197 to 235 µg for men and 168 to 214 µg for women) (52), so that a large proportion of the general population fails to attain the required natural dietary folate intake (53).

Vitamin B_{12} intake usually exceeds requirements. High-risk populations may still experience problems. Vitamin B_{12} deficiency in elderly people is frequently due to inadequate absorption resulting from an age-related decrease in gastric acid secretion or a slight increase in (gastric) pH, or to intrinsic factor deficiency, and may affect as many as 30 to 40% of the elderly population (34, 54). As vitamin B_{12} can only be synthesized by bacteria, animal food (fish, meat, eggs, dairy products) are the only good sources of vitamin B_{12} (55). Unlike folate, cobalamin is a relatively stable vitamin and almost all of it is left intact by food processing.

Meat, dairy products, whole grain cereals, potatoes, fruits, and vegetables are particularly rich in vitamin B_6 (56). No representative surveys of vitamin B_6 intake in Germany, Austria, and Switzerland are available. Data from the Framingham Heart Study show a significant increase in plasma homocysteine levels for vitamin B_6 intakes of less than approximately 1.4 mg/day (34). Vitamin B_6 shows greater stability than folic acid: Not more than 10 to 50% of vitamin B_6 is lost during storage and cooking (56).

Other causes of changes in plasma homocysteine

Numerous agents, drugs, diseases, and lifestyle factors have an impact on homocysteine metabolism, especially when acting as direct or indirect antagonists of cofactors and enzyme activities but also as a consequence of disulfide exchange reactions, impairment of absorption, and enzyme induction (32, 57). Most of the resultant clinically significant changes may therefore be important to interpreting the overall clinical picture. Moreover, plasma homocysteine levels are a useful indicator of the efficiency of some treatments (Table 1).

Mechanisms of Homocysteine-Mediated Vascular Damage (Atherothrombosis)

Homocysteine metabolism in cardiovascular cells relies exclusively on folate- and vitamin B_{12}-dependent remethylation since no transsulfuration has to date been demonstrated in endothelial cells of human blood vessels (58). Because of the absence of irreversible breakdown of homocysteine to cysteine, homocysteine synthesis may rapidly exceed cell export, resulting in specific cell injury to the point of cell death. Compared with other organ systems, the cardiovascular system is therefore particularly sensitive to elevated homocysteine levels (58). Hyperhomocysteinemia may alter vascular morphology, stimulate inflammation, activate the endothelium and the blood clotting cascade, and inhibit fibrinolysis. As a result, hyperhomocysteinemia is associated with the loss of endothelial anti-thrombotic function and induction of a procoagulant environment (32, 59). Most known forms of damage or injury (Table 2) are due to homocysteine-mediated oxidative stress. Chief among these are changes in the intracellular redox potential, interference with the nitric oxide system, and activation of transcription factors with stimulation of gene expression. Numerous mechanisms are supported by *in vivo* studies and models of diet-induced folate deficiency and physiological homocysteine elevation.

Methods and Sample Handling

Methods

A variety of methods are available for the quantitative determination of homocysteine in plasma. Common assay techniques are based on high-pressure liquid chromatography (HPLC) and immunological methods. These two assay methods show good concordance in patient populations but may show considerable within-subject differences. The usual approach is to determine plasma total homocysteine (tHcy), *i.e.*, the sum of free and bound homocysteine, after a reduction step. Quantitative shifts between the two fractions will therefore not show up in the reported concentration.

Table 2 Atherogenic effects of homocysteine (selection).

Effects	Hcy	Effects	Hcy
Vascular architecture		Oxidative stress	↑
Endothelial damage	↑	Production of peroxinitrite, H_2O_2, *etc.*	↑
VSMC proliferation	↑	Antioxidative enzymes (SOD, GPx)	↑
Collagen synthesis, fibrosis of media	↑	Lipid peroxidation	↑
Constrictive remodeling	↑	Chemotaxis, leukocyte adhesion	↑
Foam cell formation	↑	Leukocyte adhesion	↑
(Proliferative) fibrous plaques	↑	sICAM-1, VCAM-1	↑
Cell structure damage	↑	Chemotaxis (IL-8, MCP-1), vWF	↑
Mitochondrial damage	↑	Clotting activation	↑
ER stress	↑	Tissue factor	↑
Metalloproteinases	↑	Inactivation of protein C	↑
Elastolysis	↑	Thrombin (thrombin-antithrombin complex)	↑
HSP-70 expression	↓	D-Dimer	↑
Endothelial dysfunction	↑	Fibrinolysis	↓
NO system	↑↓	Heparin sulfate	↓
NO bioavailability	↓	Annexin II	↓
ADMA	↑	Thrombomodulin	↓
Transcription factors		PAI-1, t-PA antigen	↑
Activation of NF-κB, SREBP, PKC	↑	Prothrombin fragment F1+2	↑
Gene expression	↑↓	Inactivation of Factor Va	↓
HMG-CoA reductase	↑	Platelet aggregation	↑
Lipid biosynthesis	↑	Fibronectin (function)	↓
Inactivation of PPARα and γ	↑	COX, production of TXA_2 and TXB_2	↑

VSMC = vascular smooth muscle cell, ER = endoplasmic reticulum, HSP = heat shock protein, NO = nitric oxide, ADMA = asymmetric dimethylarginine, SREBP = sterol regulatory element binding protein, PKC = protein kinase C, PPAR = peroxisome proliferator-activated receptor, H_2O_2 = hydrogen peroxide, SOD = superoxide dismutase, GPx = glutathione peroxidase, sICAM = soluble intercellular adhesion molecule, VCAM = vascular cell adhesion molecule, IL = interleukin, MCP = monocyte chemotactic protein, vWF = von Willebrand Factor, PAI-1 = plasminogen activator inhibitor 1, t-PA = tissue plasminogen activator, COX = cyclooxygenase, TXA_2 = thromboxane A_2.

Quality control

While there is sufficient concordance in differentiating between normohomocysteinemia and hyperhomocysteinemia, among-method variations are still unsatisfactory (60). International standardization (development of a plasma standard) would be needed to improve between-laboratory comparability and to increase the quality of assay results. Participation in an inter-laboratory (round-robin) testing program for external quality assurance (*e.g.*, in a European Research Network for evaluation and improvement of screening, diagnosis and treatment of Inherited Metabolic Disorders (ERNDIM) quality assurance scheme; www.erndimqa.nl) would therefore be desirable and useful.

Sample preparation

A fasting blood sample collected into an EDTA tube should be used for the measurement of plasma homocysteine. The blood sample should be centrifuged immediately after collection to separate the plasma. If immediate centrifugation is impracticable, the blood sample can be stored on ice for not more than one hour. Failure to immediately centrifuge and separate the plasma from the blood cells causes a rapid increase in plasma homocysteine (by as much as 10% per hour) as a function of temperature and time, giving false-high readings (61). After centrifugation, homocysteine is stable in plasma (for 24 hours at room temperature, for up to 1 week in the refrigerator (4 °C), or for several months when deep-frozen (–20 °C)).

Serum samples should not be used because serum cannot be separated by centrifugation before the blood sample has coagulated completely. Collection tubes anticoagulated with substances other than EDTA have been used on various occasions to increase the time to centrifugation. However, as the comparability of those assay results with the readings obtained with the method described here is quite limited, the practice of immediate plasma separation by centrifugation or brief storage on ice should be followed if at all possible for the sake of better comparability of results.

Intra-individual variability

Intra-individual variability of homocysteine is very low. Repeat measurements after 6 to 18 months in healthy volunteers show good reproducibility of baseline levels with nonsignificant intra-individual variations of as little as 0.85 to 1.2 µmol/l (62, 63). Despite the low variability of homocysteine assays, repeat measurements can improve the diagnostic reliability within the range where a decision to treat or not to treat is to be made. One-time measurements, on the other hand, tend to underestimate actual risk by approximately 10 to 15% because of the associated misestimate of the true set-point (64). Without appropriate correction, risk is underestimated by approximately 20% after 2 years and approximately 50% after 10 years (64). This regression dilution increases with time, and a correction formula should be used for appropriate risk estimation in prospective clinical trials (64). This also explains why various prospective studies have tended to underestimate relative risk compared with retrospective studies (65).

Oral methionine loading

Plasma homocysteine levels are measured before, and 4 or 6 hours after, oral methionine loading (100 mg of methionine per kg of body weight). The value measured after methionine loading mainly reflects CBS activity or vitamin B_6 availability. The fasting plasma homocysteine concentration determined without methionine loading, on the other hand, is not a good indicator of vitamin B_6 deficiency (23). Subjects with a fasting plasma homocysteine between 12 and 15 µmol/l often have an abnormal oral methionine loading test (>38 µmol/l) (12). Oral methionine loading can identify more subjects with hyperhomocysteinemia than determination of fasting plasma homocysteine alone (12). However, there are currently no generally accepted criteria for interpreting methionine loading test results so that this test can as yet not be recommended for use as a routine diagnostic tool; its use should rather be reserved for (clinical) research studies.

High-Risk Populations and Plasma Homocysteine Risk Ranges

High-risk populations and profiles

Moderate hyperhomocysteinemia (plasma homocysteine concentration > 12–30 µmol/l) is found in 5 to 10% of the general population and in up to 40% of patients with vascular disease (32, 34, 61). Hyperhomocysteinemia is associated with an increased risk of atherothrombotic diseases. The determination of plasma homocysteine should therefore be part of the individual risk profile for patients with cardiovascular disease. Synergistic interactions of homocysteine with additional risk factors (smoking, arterial hypertension, diabetes, hyperlipidemia) produce an over-proportional increase in total risk; the identification of subjects/patients at high risk of vascular disease is therefore of particular importance (12, 17, 18). These target populations are likely to derive particular benefit from homocysteine-lowering treatments. Once diagnosed, patients with diabetes mellitus or metabolic syndrome should be treated like those with vascular manifestations. Individuals with a family history of cardiovascular disease/events are very likely to suffer manifestations of vascular disease at some point in their lives. Early screening for hyperhomocysteinemia is recommended for close relatives of patients in such high-risk populations. About 50% of men over the age of 40 years and about 33% of women over the age of 40 years will develop CAD (66). This is why the plasma homocysteine concentration should be known also in apparently healthy individuals at the age of 50 years at the latest. Other target populations for plasma homocysteine screening include people at (increased) risk

Table 3 Plasma homocysteine assay targets populations based on risk.

Manifest vascular disease	Populations at risk of cardiovascular disease	Populations at risk of vitamin deficiency
Coronary artery disease	Family history of CAD	Elderly people
Myocardial infarction	Arterial hypertension	Vegetarians
Carotid artery atherosclerosis	Smoking habit	Inflammatory gastrointestinal conditions (gastritis, mal absorption)
Peripheral arterial occlusive disease	Hyperlipidemia	Preeclampsia
Cerebral artery atherosclerosis	Renal insufficiency	Kidney disease
Stroke	Diabetes	Alcohol abuse
Venous thrombosis	Metabolic syndrome	Unbalanced diet
Pulmonary artery embolism		Drugs (see Table 1)

Table 4 Classification of plasma homocysteine levels by need to treat.

Homocysteine level	Classification	Treatment
> 12 to 30 µmol/l	Moderate hyperhomocysteinemia	Intervention required for all (apparently healthy individuals and patients)
10 to 12 µmol/l	Tolerable (in healthy subjects)	Need to treat patients at increased risk
< 10 µmol/l	Safe	No need to treat (target level of intervention)

for developing (atherothrombotic) vascular complications and vitamin deficiencies (Table 3).

Plasma homocysteine risk ranges

It is not helpful to specify reference ranges in the usual sense because plasma homocysteine levels below 10 µmol/l are already associated with a graded increase in risk or manifestations of cardiovascular disease (dose-response relationship) (11, 22, 67): Each 1 µmol/l increment in plasma homocysteine concentration is associated with a 6 to 7% risk increase (68).

However, differentiated prophylactic and therapeutic risk ranges for cardiovascular disease can be defined for clinical practice. For the sake of simplification, plasma homocysteine levels > 12 µmol/l and < 30 µmol/l are traditionally referred to as "moderate hyperhomocysteinemia" (commonly found in people with vitamin deficiency); the range from 30 to 100 µmol/l has been termed "intermediate hyperhomocysteinemia" (often found in individuals with homozygous enzyme defects as well as in patients with chronic kidney disease); and plasma homocysteine concentrations > 100 µmol/l are traditionally defined as "severe hyperhomocysteinemia" (typically found in individuals with severe congenital disorders or homocystinuria) (69, 70) (Table 4).

Goals of Intervention

Prophylaxis

While there is clearly an overall need to improve folic acid intake by the general population, there is currently no cogent evidence from cardiovascular outcome studies that would justify the definition of general guidelines for vitamin supplementation in apparently healthy subjects and low-risk individuals. Vitamin supplementation continues to be a recommendable option for prophylaxis. Dosages for prophylaxis are given in Figure 2 (low-dose supplementation: folic acid, 0.2 to 0.8 mg/day; vitamin B_{12}, 3 to 100 µg/day; vitamin B_6, 2 to 25 mg/day). Also, everyone is recommended to eat a diet rich in vitamins.

Although the results of ongoing intervention studies on the impact of B-vitamin supplementation on mortality reduction are not yet available, plasma homocysteine reduction (through dietary supplementation) should also be considered in apparently healthy individuals at increased risk, and especially in patients with vascular disease, because secondary endpoint evidence from previous intervention studies suggests potential benefit.

Treatment

Consistent with other working parties and consensus groups, we recommend a target plasma homocysteine level of < 10 µmol/l for patients with manifest vascular disease and high-risk individuals (16, 53, 71–75) (Figure 2). Renal impairment and thyroid dysfunction as well as vitamin deficiency should be ruled out as the cause of hyperhomocysteinemia in individuals with plasma homocysteine levels > 12 µmol/l. The factors listed in Table 1 should always be considered when interpreting findings. Thus, a significant reduction in plasma homocysteine can often be achieved by merely switching medications, making a dosage adjustment, or starting treatment of hypothyroidism.

Folate Requirements and Therapeutic Options

An adequate intake of at least 400 µg of folate per day is difficult to maintain even with a balanced diet, and high-risk groups often find it impossible to meet these

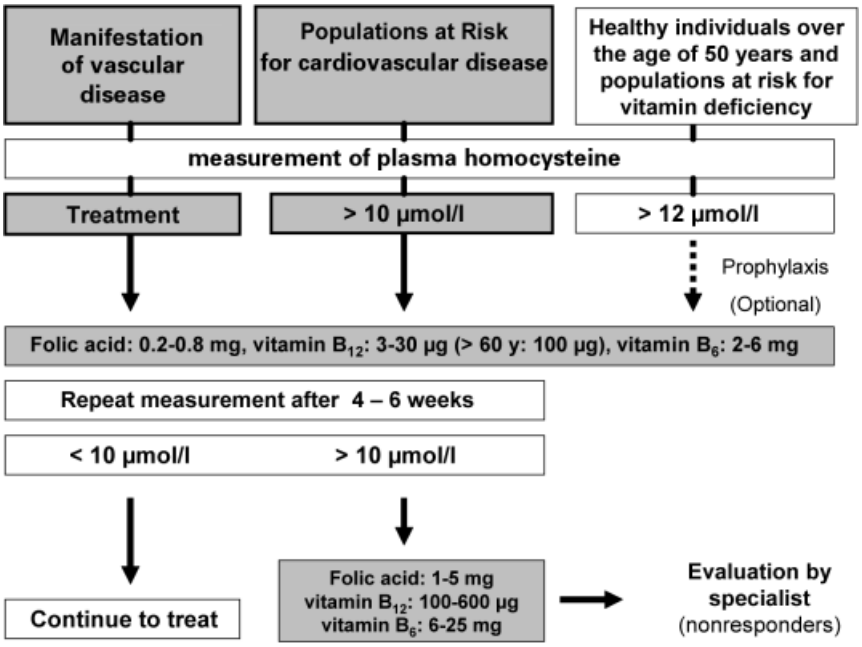

Figure 2 Decision tree for the diagnosis and prophylaxis/treatment of hyperhomocysteinemia (does not apply to patients with renal impairment).

folate requirements (52, 53). As the recommendation to eat a healthy diet has little or limited impact on elevated homocysteine levels, (folate)-fortified foods and/or vitamin supplements are rational and therefore recommended (53, 54). Maintaining a total folate intake of 600 to 650 µg/day, say, by supplementing 400 µg/day, may help lower elevated homocysteine levels; this is usually easy to achieve with fortified foods and/or vitamin supplements (52).

The bioavailability of dietary folates is 55%. The recommended daily amounts (RDAs) of the German, Austrian, and Swiss nutrition societies are based on a polyglutamate to monoglutamate ratio of 60:40 and a bioavailability of 20% for polyglutamates and 100% for monoglutamates. The extent of absorption of synthetic folic acid added to foods is 90 to 95% and that of folic acid in supplement tablets is almost 100%. As the bioavailability of synthetic folic acid is about twice that of naturally occurring folates, RDAs are given in terms of DFE: 1 µg of DFE is equivalent to 1 µg of dietary folate or 0.5 µg of synthetic folic acid (54, 76, 77).

Recommendations for Vitamin Supplementation

The absolute and relative reductions in plasma homocysteine that can be achieved with vitamin supplementation depend on the baseline homocysteine concentration and are greater for higher baseline levels. Supplementation with 0.2 to 5 mg of folic acid per day is expected to lower plasma homocysteine by 16 to 39% (the average reduction for a standardized baseline concentration of 12 µmol/l is approximately 25%) (78). Additional supplementation with vitamin B_{12} is recommended to avoid relative folate deficiency, *i.e.*, to support folate utilization (because folate is "trapped" as methyltetrahydrofolate in relative vitamin B_{12} deficiency) (31). Vitamin B_{12} supplementation is also recommended for prevention of neurodegenerative damage, a particular problem especially among elderly people. Based on these considerations, (long-term) supplementation of folic acid alone is discouraged. Instead, folate supplementation should always be combined with vitamin B_{12} supplementation. Vitamin B_6 has little impact on fasting plasma homocysteine levels, but it is an important cofactor in catabolic transsulfuration and, therefore, should be supplemented as well. Body (folate) stores are quite limited. Vitamin supplementation therefore needs to be administered chronically. Once folate (+ vitamin B_{12} + vitamin B_6) supplementation is stopped, plasma homocysteine is bound to rise again.

Supplementation in Moderate Hyperhomocysteinemia

If plasma homocysteine determination suggests moderate hyperhomocysteinemia, a repeat measurement after 4 to 6 weeks may be useful to confirm the diagnosis. Once (moderate) hyperhomocysteinemia is established, vitamin supplementation should be started, supplementing 0.2 to 0.8 mg of folic acid, 3 to 100 µg of vitamin B_{12} (elderly people should receive at least 100 µg because of malabsorption), and ideally also 2 to 25 mg of vitamin B_6. If this supplementation regimen lowers plasma homocysteine to <10 µmol/l within 4 weeks, repeat measurements of plasma homocysteine should be obtained first every 6 months and later on once a year. If response (*i.e.*, plasma homocysteine reduction) is still inadequate, the dosage of folic acid should be increased to, say, 1 to 5 mg of folic

acid per day (while supplementation with vitamin B_{12} and vitamin B_6 can be continued unchanged for some time). Repeat determinations of plasma homocysteine should be performed at 4-week intervals.

Possible other Causes of Increased Vitamin Requirements

If plasma homocysteine fails to be adequately lowered despite adequate vitamin supplementation, the patient should be evaluated for vitamin deficiency, renal impairment, and thyroid dysfunction. It should be borne in mind that a (low) "normal" vitamin B_{12} level does not rule out intracellular vitamin B_{12} deficiency. Serum methylmalonic acid and holotranscobalamin II levels are more reliable markers of vitamin B_{12} deficiency than is the serum vitamin B_{12} concentration (79, 80). Mutations of the genes encoding the enzymes involved in homocysteine metabolism may also result in increased vitamin requirements. The best known example is the MTHFR 677C→T polymorphism. Other possible causes of hyperhomocysteinemia are listed in Table 1. The determination of specific metabolites, including methylmalonic acid, 2-methylcitric acid, cystathionine, cysteine, and glutathione, may provide additional information about the type of disorder present in a particular patient.

Supplementation in Patients with Renal Dysfunction and Enzyme Deficiency

Patients with overt renal failure (insufficiency, dialysis) may require very large vitamin doses (including 3 g of betaine/day) and still fail to achieve normal plasma homocysteine levels. Patients with no renal impairment whose plasma homocysteine is >30 µmol/l may have some form of congenital enzyme deficiency whose prevalence has been underestimated in the past. If pharmacologic doses of, say, 1 to 5 mg of folic acid, 1 mg of vitamin B_{12}, and >20 mg of vitamin B_6 fail to achieve normalization of plasma homocysteine, the patient should be referred to a specialist for further evaluation.

Safety

The toxicity of folic acid is extremely low even after prolonged use of high doses. Thus, 10 mg/day administered for 5 years has been tolerated without adverse reactions (81). Higher doses have, in isolated instances, been associated with gastrointestinal symptoms, insomnia, irritability, excitation, and depression. Because of the theoretical risk of masking megaloblastic anemia and causing irreversible neurological disorders, high doses of folic acid should not be administered alone without ruling out underlying vitamin B_{12} deficiency beforehand, especially in elderly people (81). This is why the United States Food and Nutrition Board (FNB) has defined a tolerable upper intake level (UL) of 1 mg/day of folic acid, which is considered safe even with life-long supplementation. Vitamin B_{12} has for decades been used for the treatment of pernicious anemia, mainly by the parenteral (intravenous or intramuscular) route. In this indication, patients receive standard single doses of several hundred micrograms, often for the rest of their lives. Based on this extensive therapeutic experience, vitamin B_{12} (cyanocobalamin and hydroxocobalamin) can be considered non-toxic. The FNB, therefore, has not specified a UL for vitamin B_{12}. Vitamin B_6 has for many years been used in the treatment of a number of conditions, and even very high doses are usually well tolerated. A UL of 100 mg/day would not be expected to be associated with side effects even with life-long use (82). Vitamin B_6 supplementation in mild hyperhomocysteinemia typically only involves supplementation with 2 to 20 mg, and there is rarely a need to use doses of 100 mg and above.

Cost/Benefit Assessment

The accepted response-to-injury model of the development of atherosclerosis inherently includes the concept of reversibility through control of the agent(s) that cause(s) the injury (4, 6, 83). There is clearly much greater prevention potential although the prevalence of cardiovascular disease is bound to increase dramatically as a consequence of the steadily increasing life expectancy (1, 2, 7, 83, 84). Effective reduction of elevated plasma homocysteine levels by 3 to 5 µmol/l through vitamin supplementation might reduce the relative risk of cardiovascular disease by approximately 10% in the general population and by as much as 25% in high-risk groups (14, 54). This prevention potential is clearly supported by epidemiological data (85–88) and numerous favorable study results already available for secondary endpoints, including improvement in endothelial function in healthy individuals (89) and patients (90–92), slowing of progression of (carotid artery) atherosclerosis (93), and substantial reduction of the rate of coronary restenosis following percutaneous transluminal coronary angioplasty (PTCA) (94, 95). More indirect evidence of the efficacy of plasma homocysteine reduction is provided by the observation that, in the first year after the introduction of food fortification with folic acid (140 (µg)/100 g) in the United States, there were 26,696 fewer deaths from myocardial infarction and stroke (compared with 1997) (96). A most recent report (H. Lange, personal communication, ACC Chicago, 2003) discourages vitamin supplementation following coronary stent implantation for the time being.

Such an inexpensive and potentially effective intervention is rarely available for reducing morbidity, mortality, and associated costs (13, 97). For all approaches to improving vitamin intake, variable yet invariably conservative calculation models have demonstrated a very favorable cost/benefit ratio (13, 97–100). Cost-ef-

fectiveness analyses are greatly dependent upon the defined baseline risk. The current most efficient approach is therefore to screen and treat high-risk groups (97, 98). Maximum cost-effectiveness has also been calculated for screening all men over the age of 45 years (and women over the age of 55 years) with no known vascular disease and treatment of individuals with plasma homocysteine levels >10 µmol/l (13, 97). Most models do not take account of synergistic savings although diseases and their treatment/prevention should, in fact, not be treated as isolated entities (4, 13, 97). Thus, the reduction of CAD incidence would also include a reduced risk of cost-intensive conditions of old age, such as senile dementia and stroke, which account for about 30% of healthcare costs in over 85-year-olds. In addition to the cited cardiovascular disease prevention potential, improved folate and vitamin B_{12} intake/supplementation is likely to have preventive effects on congenital malformations/birth defects, malignant disease, pernicious anemia, depression, and Alzheimer's disease. Further position papers are planned to address these issues.

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