Preventive health care, 2000 update: screening and management of hyperhomocysteinemia for the prevention of coronary artery disease events

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Abstract

Objective: To establish guidelines for the screening and treatment of hyperhomocysteinemia in the investigation and management of coronary artery disease (CAD).

Options: Measurement of plasma total homocysteine (tHcy) levels in the fasting state or 4–6 hours after oral methionine load; vitamin supplementation with folic acid and vitamins B_6 and B_{12}; adherence to the recommended daily allowance of dietary sources of folate and vitamins B_6 and B_{12}.

Outcomes: This article reviews the available evidence on the association between plasma tHcy levels and CAD and the effect of lowering tHcy levels through vitamin supplementation or dietary intake.

Evidence: MEDLINE was searched for relevant English-language articles published from January 1966 to June 1999; also reviewed were additional articles identified from the bibliographies.

Benefits, harms and costs: Cardiovascular disease is the leading cause of death in Canada. Homocysteine, generated in the metabolism of methionine, may have a role in the development of cardiovascular disease. The prevalence of hyperhomocysteinemia in the general population is between 5% and 10% and may be as high as 30%–40% in the elderly population. If population-based studies are correct, tHcy may be responsible for up to 10% of CAD events and thus may represent an important and potentially modifiable risk factor for cardiovascular disease. Laboratory testing for tHcy is currently restricted to research centres, and costs range from $30 to $50 per person. Newer, less costly techniques have been developed and should become readily available with time.

Values: The strength of evidence was evaluated using the methods of the Canadian Task Force on Preventive Health Care.

Recommendations: Although there is insufficient evidence to recommend the screening or management of hyperhomocysteinemia at present (grade C recommendation), adherence to recommended daily allowance of dietary sources of folate and vitamins B_{12} and B_6 should be encouraged. If elevated tHcy levels are discovered, vitamin deficiency should be ruled out to allow specific treatment and prevention of complications, such as neurological sequelae due to vitamin B_{12} deficiency. Experts in the field advocate treatment of elevated tHcy levels in high-risk people, such as those with a personal or family history of premature atherosclerosis or a predisposition to develop hyperhomocysteinemia. Definitive guidelines for the management of hyperhomocysteinemia await the completion of randomized trials to establish the effect of vitamin supplementation on CAD events.

Validation: The findings of this analysis were reviewed through an iterative process by the members of the Canadian Task Force on Preventive Health Care.

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Research

Recherche

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Cardiovascular disease is the leading cause of death in Canada, accounting for almost 40% of all deaths.[1] Although rates of death from ischemic heart disease are declining,[2] the costs to society remain high. Since a number of cardiovascular deaths may be preventable, the search for novel risk factors continues. Homocysteine is an intermediate that is generated in the metabolism of methionine (Fig. 1). Several intriguing observations suggest a role for homocysteine in the development of vascular disease. People who have homocystinuria,[3] an autosomal recessive disorder, have severe hyperhomocysteinemia, premature atherosclerosis and thromboembolic complications.[4] Furthermore, homocysteine may promote the oxidation of low-density lipoprotein cholesterol, vascular smooth muscle cell proliferation, platelet and coagulation factor activation, and endothelial dysfunction.[5–7] Therefore, altered homocysteine metabolism has become the focus of increasing attention because of its potential role in the pathogenesis of atherosclerosis and other conditions, such as venous thrombosis.[8,9]

The prevalence of hyperhomocysteinemia in the general population is between 5% and 10%, according to a threshold set at the 90th or 95th percentile (about 15 μmol/L).[7] However, rates may be as high as 30%–40% in the elderly population.[10] If the results from population-based studies are correct, then up to 10% of events due to coronary artery disease (CAD) may be attributable to elevated plasma homocysteine levels.[11] Thus, homocysteine may represent an important and potentially modifiable risk factor for cardiovascular disease.

The purpose of this review was to evaluate the quality of evidence pertaining to homocysteine and CAD events and to make recommendations regarding the screening and management of hyperhomocysteinemia.

Methods

A computerized search of MEDLINE for English-language articles published between January 1966 and June 1999 was conducted using the MeSH (medical subject heading) terms "homocysteine," "hyperhomocysteinemia," "methionine," "coronary disease," "arteriosclerosis," "myocardial ischemia," "folic acid," "vitamin B_{12}," "vitamin B_6," and "pyridoxine" in various combinations. Relevant articles were also identified through a manual review of references. Where possible, the highest level of evidence was sought; hence, abstracts, cross-sectional studies, case reports and case series were not included. Studies concerning other types of vascular disease were also excluded.

The evidence was reviewed systematically using the methodology of the Canadian Task Force on Preventive Health Care. The task force, comprised of expert clinicians and methodologists from a variety of medical specialties, used a standardized evidence-based method (Appendix 1) for evaluating the effectiveness of this intervention. The final recommendations were arrived at unanimously by an expert panel and principal author. Feedback from 2 content experts was incorporated into a final draft of the manuscript before submission for publication. Procedures to achieve adequate documentation, consistency, comprehensiveness, objectivity and adherence to the task force's methodology were main-

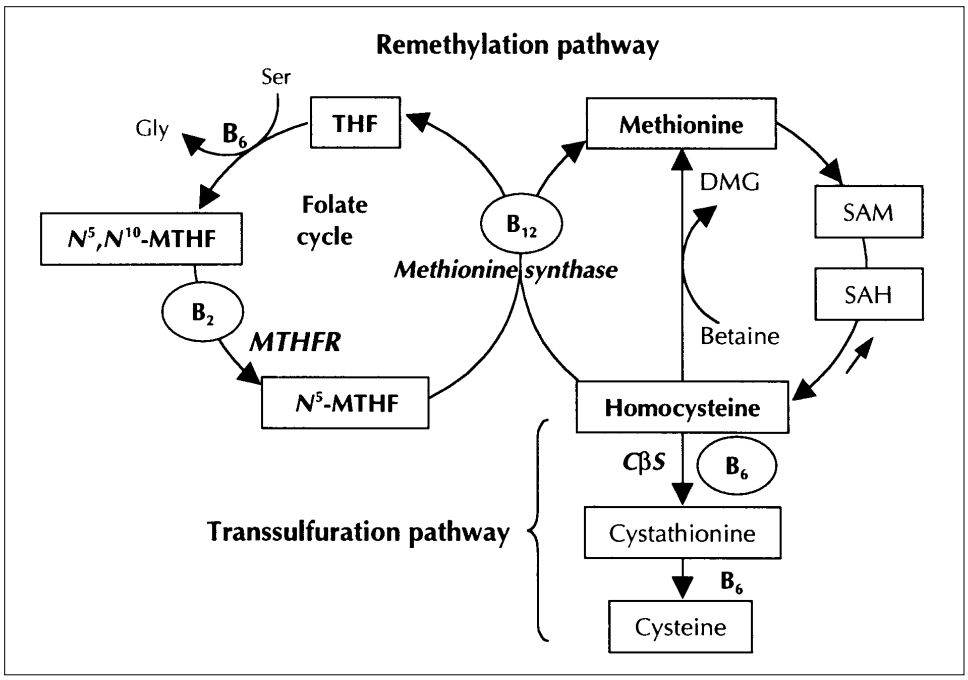

Fig. 1: Biochemical pathways of homocysteine metabolism. Ser = serine; Gly = glycine; MTHF = methylenetetrahydrofolate; MTHFR = N^5,N^{10}-methylenetetrahydrofolate reductase; THF = tetrahydrofolate; SAM = *S*-adenosylmethionine; SAH = *S*-adenosylhomocysteine; DMG = dimethylglycine; CβS = cystathionineβ-synthase.

tained at all stages during review development, the consensus process and production of the final manuscript. The full methodology has been described previously.[12]

Screening tests for hyperhomocysteinemia

Most assays measure levels of total homocysteine (tHcy) (protein-bound and complexed moieties)[13] in the fasting state or 4–6 hours after an oral methionine load (0.1 mg/kg).[7] High-pressure liquid chromatography, the most common method, has a coefficient of variation of 3%–11%.[13] Samples must be placed immediately on ice to avoid spurious elevations in tHcy levels.[7] Furthermore, levels are falsely low in the acute phase of illness such as myocardial infarction (MI).[14] Current screening tests cost between $30 and $50; however, newer, less costly techniques for measuring tHcy levels have been developed[15] and should become readily available with time.

Aside from genetic predisposition, a number of factors raise plasma tHcy levels, including increasing age, male sex and elevated serum creatinine levels.[5–7] Drugs such as antiepileptic agents, methotrexate and nitrous oxide[6] and certain disease states such as psoriasis, acute lymphoblastic leukemia, breast cancer and hypothyroidism[5,6] also increase tHcy levels, likely through effects on vitamin status. Homocysteine is inversely correlated with serum vitamin B_6, vitamin B_{12} and folate levels.[10] Thus, in populations with a higher prevalence of vitamin B_{12} deficiency, such as elderly people,[16] the specificity of plasma tHcy as a cardiac risk factor may be reduced.

Association between homocysteine and risk of CAD events

Retrospective studies

Over 30 case–control studies have compared tHcy levels between CAD patients and healthy control subjects.[17–46] Patients with CAD had significantly higher fasting plasma tHcy levels in 22 of 27 studies[18–39] (odds ratio [OR] 1.2–10.9 after adjustment for other cardiac risk factors). Levels after methionine load were also higher in patients with CAD in 8 of 9 studies[35–42] (adjusted OR 1.3–6.7).[35,43] Measurement of serum levels yielded similar results.[17,40] Moreover, 2 meta-analyses of retrospective data[11,47] confirmed these findings: the odds ratios of CAD associated with elevated plasma tHcy levels were 1.7 (95% confidence interval [CI] 1.5–1.9) and 6.14 (95% CI 2.74–13.73). The relation between tHcy levels and the number of occluded coronary vessels was not consistent.[17,30–32]

It is possible that some other factor predisposes to both CAD and hyperhomocysteinemia. As anticipated, cardiac risk factors were more common among patients with CAD. Volunteer bias may exaggerate this difference because participants of clinical trials tend to be healthier and thus control subjects may have fewer risk factors than do people in the general population. Certain healthy behaviours, such as low caffeine intake[48] and multivitamin use,[10,35] are inversely associated with plasma tHcy levels. Homocysteine may be related to risk factors such as smoking,[35,37] hypertension,[32–35] dyslipidemia[27–29,35] and hyperglycemia;[49] however, it appears to have an independent effect[33–38] and may even interact with other factors to influence CAD risk.[19,35] In one study adjustment for plasma fibrinogen abolished the association between tHcy and CAD.[17] The relation between tHcy and other unconventional risk factors is unknown. Thus, retrospective studies can show an association but not a causal relation.

Prospective studies

Eight nested case–control studies prospectively evaluated the relation between tHcy and the occurrence of a first major CAD event[47,50–55] or new-onset angina necessitating coronary artery bypass surgery.[56] Unfortunately, these studies had conflicting findings. MI and coronary death were associated with higher tHcy levels in only 4 of 7 studies,[47,50–52] and adjustment for prevalent CAD attenuated this relation in 1 study.[52] In the MRFIT trial,[54] a minority of patients who had early CAD events had sufficient frozen plasma available to measure tHcy levels. Thus, in many cases, plasma tHcy may have been measured too far in advance. In

Table 1: Relation between plasma total homocysteine (tHcy) levels and overall mortality and death due to coronary artery disease (CAD)

Plasma tHcy level, μmol/L	No. of subjects n = 587	Overall mortality, %	Relative risk (and 95% CI)	
			Death*	CAD-related death†
< 9	130	3.8	1.0	1.0
9–14.9	372	9.9	1.9 (0.7–5.1)	2.3 (0.7–7.7)
15–19.9	59	25.4	2.8 (0.9–9.0)	2.5 (0.6–10.5)
≥ 20	26	26.9	4.5 (1.2–16.6)	7.8 (1.7–35.1)

Source: abridged from Nygård et al.[60]
Note: CI = confidence interval.
*Adjusted for age, sex, left ventricular ejection fraction, creatinine level, total cholesterol level, extent of CAD, treatment for hypertension, history of diabetes mellitus, smoking history, platelet count and use of ASA.
†Adjusted for age, sex, left ventricular ejection fraction, creatinine level, total cholesterol level and extent of CAD; p for trend = 0.01.

contrast to major CAD events, the development of angina was not related to plasma tHcy.[56]

Prospective cohort studies suggest that tHcy is a greater risk factor for major CAD events in patients with established CAD. In 2 studies[57,58] coronary events occurred more frequently in men with hyperhomocysteinemia than in men with normal tHcy levels; however, adjustment for prevalent CAD at baseline attenuated this association. In contrast, nonfasting tHcy levels were independently related to death due to cardiovascular disease (relative risk [RR] 1.52, 95% CI 1.16–1.98) and overall mortality (RR 1.54, 95% CI 1.31–1.82) in elderly men and women from the original Framingham cohort.[59] The most compelling evidence comes from Nygård and associates,[60] who prospectively followed 587 patients with significant stenosis on coronary angiography. A dose–response relation between baseline homocysteine levels and both death due to CAD and overall mortality was observed (Table 1). Similar findings were noted among patients with other forms of atherosclerosis.[61]

The evidence from the prospective studies suggests that homocysteine acts by promoting acute thromboembolic events. Few prospective studies have evaluated the role of homocysteine in the chronic progression of atherosclerosis. In the Shunt Occlusion Trial[62] graft occlusion rates 1 year after coronary artery bypass surgery were not related to preoperative tHcy levels. However, atherosclerotic changes incurred by tHcy may require longer than 1 year to develop. In summary, the evidence strongly suggests that plasma tHcy is a risk factor for acute cardiac events in patients with underlying vascular disease.

Association between genetic predisposition to hyperhomocysteinemia and CAD

Homozygosity for a mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, involved in homocysteine metabolism, is found in 4%–14% of the general population[2] and is associated with elevated plasma tHcy[63–67] levels under conditions of impaired folate status.[63–66] The importance of this mutation in the development of CAD may depend on the population. Studies involving Japanese people have consistently shown a higher prevalence of the mutation among patients with CAD,[68–71] whereas only a minority of studies involving whites detected an association.[24,36] Moreover, a relation between the MTHFR genotype and the number of occluded vessels on coronary angiography was observed in Japanese patients[68] but not in whites.[72–74] No association between the mutation and other risk factors, including a family history of premature CAD, has been shown.[73–75]

In one meta-analysis, involving almost 5000 patients from 8 studies, the homozygous mutation was associated with an increased risk of CAD (OR 1.22, 95% CI 1.01–1.47).[67] However, a larger meta-analysis of 23 studies failed to demonstrate an association between the MTHFR genotype and either CAD (OR 1.11, 95% CI 0.91–1.37) or any cardiovascular end point (OR 1.12, 95% CI 0.92–1.37).[76] A Japanese study found a declining prevalence of the MTHFR mutation with increasing age; this finding suggests that the mutation may lead to early deaths due to cardiovas-

Table 2: Summary table of recommendations (screening and treatment of hyperhomocysteinemia)

Manoeuvre	Effectiveness	Level of evidence*	Recommendation*
Screening for plasma tHcy level†	Currently available techniques for measuring tHcy levels have a coefficient of variation of 2%–11%. Testing is restricted to research centres		
General population	An association between tHcy levels and CAD risk has been shown (the majority of studies measured fasting tHcy levels). However, the effect of screening on patient outcomes is unknown	Cohort[59] and case–control[17–42,47,50–52] studies (II-2)	Insufficient evidence to recommend for or against screening for hyperhomocysteinemia in the general population (grade C)
People at high risk for CAD events	Prospective studies have shown a more consistent relation between tHcy levels and CAD events in patients with pre-existing CAD. Again, the effect of screening on patient outcomes is unknown	Cohort[57–61] and case–control[17–42,50,52] studies (II-2)	Insufficient evidence to recommend for or against screening for hyperhomocysteinemia in high-risk populations (grade C)‡
Vitamin therapy	Treatment with folic acid (alone or with vitamin B_{12}) is effective in lowering plasma tHcy levels, whereas vitamin B_6 lowers post-methionine load levels. There are no completed studies regarding the effectiveness of treatment on clinical outcomes	RCTs[89,96–99] (I), cohort study[100] (II-2) and uncontrolled studies[90–95,101] (II-3)	Insufficient evidence to recommend for or against treatment of hyperhomocysteinemia with vitamin therapy (grade C)§

Note: RCTs = randomized controlled trials.
*See Appendix 1 for definitions of the levels of evidence and grades of recommendations.
†In fasting state or after methionine load.
‡Screening may identify individuals at higher risk of developing coronary artery disease, leading to aggressive risk factor modification. However, there is insufficient evidence to recommend screening for the purpose of treating hyperhomocysteinemia.
§Although folic acid effectively lowers plasma tHcy levels, there is insufficient evidence to support that its use would prevent CAD events.

cular disease.[77] However, the mutation does not appear to be related to longevity in whites (OR 0.87, 95% CI 0.69–1.11).[78] In light of these observations, it has been suggested that other genetic abnormalities or risk factors may interact with the MTHFR mutation to increase cardiovascular risk.[79]

Association between serum folate, vitamin B_6 or vitamin B_{12} levels and risk of CAD events

Homocysteine may simply be a nonspecific marker of vitamin deficiency. Several studies identified an inverse relation between serum folate levels and CAD events on univariate analysis; however, adjustment for plasma tHcy levels obliterated this effect.[80–82] Serum vitamin B_{12} levels have no relation to CAD; however, vitamin B_6 may be an independent risk factor. Robinson and associates[82] observed an increased risk of CAD among patients with low vitamin B_6 levels (OR 1.84, 95% CI 1.39–2.42) that remained after adjustment for plasma tHcy levels. Similarly, homocysteine remained a significant predictor of CAD after controlling for serum vitamin levels.[60,82]

Effect of vitamin therapy

Several randomized controlled trials have evaluated the

Table 3: Dietary sources of folic acid, vitamin B_6 and vitamin B_{12}

Nutrient	Recommended daily allowance	Average dietary intake by adults	Dietary sources
Folic acid	Women: 180 μg Men: 200 μg	200–300 μg	Green leafy vegetables (e.g., spinach, broccoli), legumes (e.g., lentils, chickpeas, lima beans), orange juice, oranges, cereals, breads, wheat germ
Vitamin B_6	1.6 mg	1.5 mg	Meat, poultry, fish, green leafy vegetables, legumes, seeds, potatoes, cantaloupe, milk, egg yolks, cereals, grains, wheat, wheat germ
Vitamin B_{12}	2.4 μg	4–8 μg	Beef, poultry, fish (particularly crab, oyster, salmon and herring), liver, kidney, soy, fruit juice, dairy products, egg yolks, fortified cereals, breads

Table 4: Randomized trials of homocysteine-lowering interventions currently underway

Study	Population	Start date	Sample size
Bergen Vitamin Study	Stroke (Norway)	1997	2000
Cambridge Heart Antioxidant Study (CHAOS-2)	MI, unstable angina (United Kingdom)	1998	4000
Heart Outcomes Prevention Evaluation (HOPE-2) Study	Arterial vascular disease (Canada)	1999	5000
Norwegian Study of Homocysteine Lowering with B-Vitamins in Myocardial Infarction (NORVIT)	MI (Norway)	1998	3000
Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease (PACIFIC) Study	Arterial vascular disease (Australia)	1998	10 000
Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)	MI (United Kingdom)	1998	12 000
Vitamins in Stroke Prevention (VISP) Trial	Stroke (United States)	1998	3600
VITAmins TO Prevent Stroke Study (VITATOPS)	Stroke (Australia)	1999	5000
Women's Antioxidant and Cardiovascular Disease Study (WACS)	Vascular disease or high risk for vascular disease (United States)	1998	8000

Note: MI = myocardial infarction.
Source: abridged from Eikelboom et al.[107]

effect of multivitamin supplementation on fasting tHcy levels. All treatment regimens, including a combination of folic acid (1–5 mg), vitamin B_6 (5–50 mg) and vitamin B_{12} (0.02–1 mg), lowered fasting plasma tHcy levels by 20% to 50%.[83–88] Treatment response in patients with CAD was equivalent to that in healthy control subjects.[24] A meta-analysis[89] of 12 studies, involving a total of 1114 patients, revealed that folic acid (0.4–5 mg) lowered tHcy levels by 25% on average in people with or without vascular disease. Higher plasma tHcy levels and lower serum folate levels enhanced this effect. The addition of vitamin B_{12}, but not vitamin B_6, led to a further reduction in fasting tHcy levels of about 7% (95% CI 3%–10%). The effect of folic acid was maximal at 0.5 mg/d; however, 0.2 mg may be sufficient to normalize fasting tHcy levels in some patients.[90,91] Lower doses, such as 0.1 mg, appear to be ineffective.[91,92]

Although vitamin B_6 was found to have little effect on fasting plasma tHcy levels, uncontrolled studies have illustrated its utility in the treatment of hyperhomocysteinemia after methionine load. Post-load tHcy levels were found to decline by 21%–42% following treatment with vitamin B_6 (50–250 mg).[93,94] In patients who respond poorly to vitamin B_6 (up to 25%[94]), folic acid may be effective. In the majority of patients 6 weeks of treatment with single or combination therapy is sufficient to normalize tHcy levels.[95]

In a study involving young women folate from natural food sources or fortified foods had the same effect on plasma tHcy levels (12%–21% reduction) as equivalent doses of vitamin supplements.[96] However, in middle-aged men and women, breakfast cereal fortified with 666 μg of folic acid per day had a modest effect on fasting tHcy levels (10%–14% reduction),[97–99] regardless of whether vitamin B_6 or B_{12} or other micronutrients were added.[98] Thus, current recommendations by the Food and Drug Administration in the United States to fortify cereal grain products with 140 μg of folic acid per 100g serving may be insufficient to normalize elevated homocysteine levels.[99]

Prevention of CAD

Whether lowering plasma homocysteine levels will prevent CAD events is unknown because of the absence of longer randomized controlled trials with appropriate end points. Some epidemiological data suggest that folate and vitamin B_6 intake may influence the occurrence of major CAD events. The Nurses' Health Study[100] found that women who consumed more than 400 μg of folate or 3 mg of vitamin B_6 per day had a lower risk of heart disease than those with lower intake levels (adjusted RR 0.69 for folate, 95% CI 0.55–0.87, and 0.67 for vitamin B_6, 95% CI 0.53–0.85). Furthermore, an uncontrolled study showed that areas of plaque in carotid arteries regressed in 38 patients with hyperhomocysteinemia who were given daily amounts of folic acid (2.5–5 mg), vitamin B_6 (25 mg) and vitamin B_{12} (250 μg) over 4 years.[101] Similarly, in another study patients with homocystinuria who received a minimum of folic acid (5 mg) and vitamin B_6 (100–200 mg) experienced fewer vascular events (RR 0.09, 95% CI 0.02–0.38) than patients who remained untreated.[102] Although striking, findings from the latter study cannot be extrapolated to the general population because of patient differences and other methodological issues.

Recommendations

By the Canadian Task Force on Preventive Health Care

The task force's recommendations are summarized in Table 2. There is insufficient evidence to include or exclude screening of tHcy levels in any population (grade C recommendation). Screening may enable identification of patients at high risk for CAD so that other risk factors can be managed aggressively. However, laboratory testing for homocysteine is currently restricted to research centres. Moreover, testing is not yet covered by provincial health insurance, and therefore patients may be required to cover the cost.

Although folic acid effectively lowers plasma tHcy levels, there is insufficient evidence to suggest that its use would prevent CAD events (grade C recommendation). Adherence to the recommended daily allowance of dietary sources of folate and vitamins B_6 and B_{12} (Table 3) may prevent hyperhomocysteinemia due to vitamin deficiency. Once elevated tHcy levels are discovered, vitamin deficiency should be ruled out to allow specific treatment and prevention of complications, such as neurological sequelae due to vitamin B_{12} deficiency. Some authorities recommend limiting folic acid intake to 1 mg/d[103,104] or adding higher doses of vitamin B_{12} (0.2–1 mg/d)[105] because of the theoretical risk of unmasking occult vitamin B_{12} deficiency.[103]

By other groups

Guidelines from the American Heart Association[104] state that it may be reasonable to screen tHcy levels in people who are at risk for hyperhomocysteinemia (e.g., those with renal failure) or in those who have a personal or family history of premature atherosclerosis. Several experts in the area concur[5,105,106] and suggest lowering fasting tHcy levels to less than 10 μmol/L.[105] If initial treatment with dietary sources are ineffective, then supplements or fortified foods containing at least 400 μg of folic acid, 2 mg of vitamin B_6 and 6 μg of vitamin B_{12} can be used.

Research agenda

Large-scale randomized trials designed to assess the effect of folic acid therapy on cardiovascular events are underway[107] (Table 4). Thus, evidence on which to base recommendations for the treatment of hyperhomocysteinemia is forthcoming. The optimal vitamin dose and regimen, and the role of methionine-load testing in the diagnosis of

this disorder need to be clarified. Although folic acid attained through food sources alone may be insufficient to normalize elevated tHcy levels, people with low folate intake are more susceptible to hyperhomocysteinemia. Most flour and cereal products consumed by Canadians are produced domestically, where folic acid fortification is optional. Review of fortification policies will be necessary as our knowledge regarding prevention and treatment of hyperhomocysteinemia advances.

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Appendix 1: Canadian Task Force on Preventive Health Care levels of evidence and grades of recommendations

Levels of evidence

I	Evidence from at least one well-designed randomized controlled trial
II-1	Evidence from well-designed controlled trials without randomization
II-2	Evidence from well-designed cohort or case–control analytic studies, preferably from more than one centre or research group
II-3	Evidence from comparisons between times or places with or without the intervention; dramatic results from uncontrolled studies could be included here
III	Opinions of respected authorities, based on clinical experience; descriptive studies or reports of expert committees

Grades of recommendations

A	Good evidence to support the recommendation that the condition or manoevre be specifically considered in a periodic health examination (PHE)
B	Fair evidence to support the recommendation that the condition or manoevre be specifically considered in a PHE
C	Insufficient evidence regarding inclusion or exclusion of the condition or manoevre in a PHE, but recommendations may be made on other grounds
D	Fair evidence to support the recommendation that the condition or manoevre be specifically excluded from a PHE
E	Good evidence to support the recommendation that the condition or manoevre be specifically excluded from a PHE