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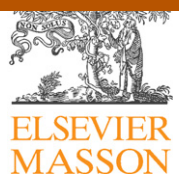

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REVIEW

The controversial role of B-vitamins in cardiovascular risk: An update

Rôle controversé des vitamines B sur le risque cardiovasculaire : mise au point

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Summary Cardiovascular disease is the leading cause of death in Western countries. Since 1969, homocysteine has been implicated in the atherosclerotic process, and numerous observational studies have suggested that hyperhomocysteinaemia should be considered as an independent cardiovascular risk factor. B-vitamins, particularly folic acid, reduce homocysteine levels effectively; it was suggested, therefore, that supplementation with these vitamins might decrease cardiovascular risk and reduce the morbidity and mortality associated with stroke, coronary heart disease and peripheral artery disease. However, the results of clinical trials conducted to investigate this issue have been inconsistent. This review discusses the findings of these trials and provides an updated overview on the 'homocysteine hypothesis'.

MOTS CLÉS
Homocystéine ;
Vitamines B ;
Acide folique ;
Risque cardiovasculaire ;

Résumé La pathologie cardiovasculaire est la première cause de décès en occident. Depuis 1969, le rôle de l'homocystéine a été impliqué dans l'athérosclérose et de multiples études observationnelles ont suggéré qu'une hyperhomocystéinémie pouvait être considérée comme un facteur de risque cardiovasculaire indépendant. Les vitamines B et en particulier l'acide folique réduisent les taux d'homocystéine, et ainsi, il a été suggéré qu'une supplémentation en vitamines pouvait réduire le risque cardiovasculaire et ainsi la morbi-mortalité liée aux accidents vasculaires cérébraux, à la maladie coronaire, et aux artériopathies périphériques. Cependant,

Abbreviations: CBS, cystathionine β-synthetase; CHD, coronary heart disease; CVD, cardiovascular disease; IMT, intima-media thickness; RR, relative risk.

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Accident vasculaire cérébral ;
Maladie coronaire

les résultats des études cliniques en cours devraient éclairer ces observations initiales, qui ne sont pas encore complètement convaincantes. Cette revue générale discute les apports et résultats des différentes études cliniques et apporte un éclairage sur les données les plus récentes concernant l'hypothèse de l'homocystéine, comme facteur de risque cardiovasculaire.

Introduction

In 1969, McCully was the first to suggest that homocysteine may be involved in the pathophysiology of the atherosclerotic process [1]. Since then, numerous observational studies have associated hyperhomocysteinaemia with cardiovascular risk, and have established homocysteine as an independent risk factor [2—21]. Folic acid and vitamins B_{12} and B_6 are important cofactors in the metabolism of homocysteine and have been shown to reduce elevated homocysteine levels effectively [22—43]. It was suggested, therefore, that supplementation with folic acid, vitamin B_{12} and vitamin B_6 might decrease cardiovascular mortality substantially — the 'homocysteine hypothesis' [44]. Several large clinical trials have been designed to test this theory. Some of them, such as the Second Cambridge AntiOxidant Heart Study (CHAOS-2), the Vitamin Intervention for Stroke Prevention (VISP) trial, the Norwegian Vitamin (NORVIT) trial, the Heart Outcome Prevention Evaluation-2 (HOPE-2) trial, the Homocysteinemia in Kidney and End-Stage Renal Disease (HOST) trial, the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS) and the Western Norway B-vitamin Intervention Trial (WENBIT) have already been published, with inconsistent results. It has been suggested that the implementation of mandatory fortification of cereals with folic acid in the United States and Canada (aiming to reduce neural tube defects) may have influenced the results of these trials [45]. In this review, we discuss the findings of these trials and attempt to provide an updated overview on the 'homocysteine hypothesis'.

Homocysteine metabolism and causes of hyperhomocysteinaemia

Homocysteine is an essential amino acid, which is derived from the conversion of methionine to cysteine. homocysteine is metabolized via two pathways (Fig. 1): remethylation, in which homocysteine is reconverted into methionine, and transulphuration, in which it is converted into cysteine. In the former pathway, homocysteine acquires a methyl group, either from the conversion of 5-methyltetrahydrofolate into hydrofolate or from the conversion of betaine into N, N-dimethylglycine. Vitamin B_{12} is an important coenzyme in the conversion of 5-methyltetrahydrofolate into hydrofolate and therefore for the remethylation pathway and the metabolism of homocysteine into methionine. In the later pathway (transulphuration), homocysteine is attached to a serine molecule and forms cystathionine with the aid of CBS and vitamin B_6, which act as an enzyme and a coenzyme, respectively. Methionine is converted into homocysteine via its conversion into S-adenosylmethionine (SAM), which then loses a methyl group and becomes S-adenosyl-homocysteine (SAH), which finally hydrolyzes into homocysteine and adenosine [46].

Serum homocysteine levels between 5 and 15 μmol/L are considered to be normal. Several conditions have been associated with hyperhomocysteinaemia (Table 1): the most frequent genetic defect is a mutation of the methylene tetrahydrofolate reductase (MTHFR) enzyme, which leads to a 677 C→T substitution and is associated with mild hyperhomocysteinaemia [47]. By comparison, homozygous deficiency of CBS is associated with severe hyperhomocysteinaemia, up to 40-fold higher than normal [48,49]. The D919G mutation in the methionine synthase (MS) gene presents with hyperhomocysteinaemia (200—400 μmol/L), mental retardation, skeletal malformations and premature atherosclerosis [50]. Age is another important factor in serum homocysteine levels, which increase with advancing age, possibly due to a decrease in the activity of CBS [51]. homocysteine is also increased in men compared with in premenopausal women; this difference is also present in postmenopausal women, albeit smaller [52]. Diet, particularly methionine intake, is directly associated with homocysteine levels. As a result, homocysteine levels are low in people with low intake of animal proteins. In contrast, homocysteine levels are inversely related to vitamin intake and tend to be lower in people with a diet rich in fruits and vegetables [53]. Alcohol increases

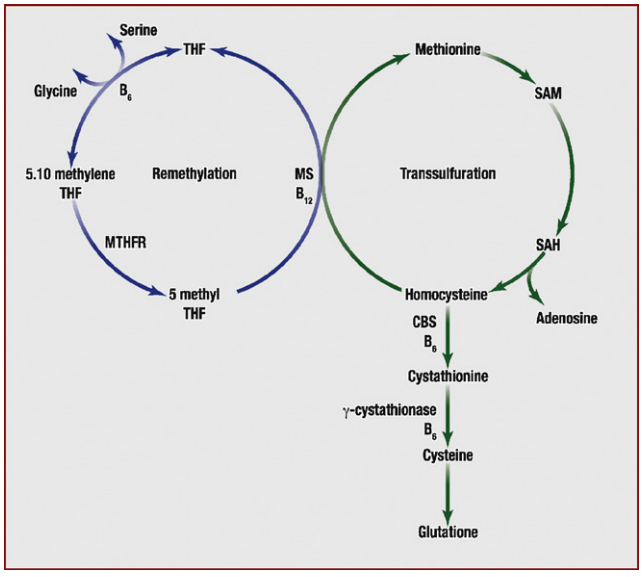

Figure 1. Homocysteine metabolism. THF: tetrahydrofolate; MTHFR: methylene tetrahydrofolate reductase; MS: methionine synthetase; SAM: S-adenosyl methionine; SAH: S-adenosyl homocysteine; CBS: cystathionine β-synthetase.

Table 1 Causes of hyperhomocysteinaemia.

Methylene tetrahydrofolate reductase polymorphism	Renal insufficiency
677 T → G	Kidney transplantation
1298 A → C	Leukaemia
1317 T → C	Psoriasis
Cystathionine β-synthase polymorphism	Sickle-cell anaemia
833 T → C	Polycythaemia vera
919 G → A	Idiopathic thrombocytosis
1730 G → A	Drugs
Methionine synthetase polymorphism D919G	Fibrates
Folic acid deficiency	Folic acid antagonists
Vitamin B_{12} deficiency	Cyclosporine
Vitamin B_6 deficiency	Methotrexate
Increasing age	L-dopa
High protein intake	Antiepileptics
Low intake of vegetables/fruits	Metformin
Alcohol	

serum homocysteine, possibly due to interference with folate metabolism [53]. Several drugs cause hyperhomocysteinaemia, such as cyclosporine [53], methotrexate [53], fibrates [54] and L-dopa [55]. Serum homocysteine is directly associated with renal function and glomerular filtration rate; patients with end-stage renal disease (ESRD) present with significant hyperhomocysteinaemia [56,57]. Other causes of hyperhomocysteinaemia include leukaemia [58], psoriasis [59], sickle-cell anaemia [60], polycythaemia vera and idiopathic thrombocytosis [61].

The homocysteine hypothesis

In 1969, McCully was the first to associate hyperhomocysteinaemia with atherosclerosis when he studied the cases of two children who presented with several atherosclerotic lesions and homocystinuria, and died due to ischaemic stroke [1]. Since then, numerous observational studies have associated hyperhomocysteinaemia with all facets of atherosclerotic disease, such as ischaemic stroke, CHD and peripheral artery disease [2–21]. In 1995, the meta-analysis by Boushey et al. concluded that a 5 μmol/L increase in homocysteine levels is associated with a relative risk of 1.5 (95% confidence interval [CI] 1.3–1.9) for ischaemic stroke and 1.6 (95% CI 1.4–1.7) and 1.8 (95% CI 1.3–1.9) for CHD in men and women, respectively [62]. Similar findings were also reported in the meta-analysis by Wald et al. in 2002 [63]. The meta-analysis of the Homocysteine Studies Collaboration showed that a 3 μmol/L decrease in serum homocysteine was linked with a 24% reduction in the incidence of ischaemic stroke and a 16% reduction in the incidence of CHD, whereas a 25% reduction of homocysteine levels reduced these risks by 19% and 11%, respectively [2]. All these data provided strong evidence that homocysteine should be regarded as an independent risk factor for atherosclerotic CVD.

The next challenge was to determine the most efficient way to lower homocysteine levels. Numerous interventional studies have investigated the role of B-vitamins (B_6, B_{12} and folic acid) in this regard [22–43]. In 2005, the Homocysteine Lowering Trialists' Collaboration meta-analysis included 25 randomized trials and 2596 patients to test the efficacy of B-vitamins [64]. It was shown that 0.2 mg of folic acid was associated with a 13% (95% CI 10–16) reduction in homocysteine levels over a mean period of 8 months, whereas it reached a 25% (95% CI 22–28) reduction when 5 mg of folic acid were administered. Vitamin B_{12} offered a further 5% reduction in homocysteine levels, whereas vitamin B_6 had no significant effect. The beneficial effect of folic acid on serum homocysteine levels was further confirmed by Jacques et al., who reported that mean homocysteine concentration and prevalence of high homocysteine concentrations (> 13 μmol/L) decreased from 10.1 to 9.4 μmol/L and from 18.7% to 9.8%, respectively, after the implementation of mandatory fortification of cereals with folic acid in the United States in 1998 [65].

Vitamin supplementation: what is the evidence?

Large outcome trials

In the late 1990 s, several large-scale, randomized clinical trials were designed and initiated to assess the effect of B-vitamin supplementation on cardiovascular mortality and morbidity [66]. These trials have different characteristics in terms of the population studied (fortified or not), the daily dose of B-vitamins, concomitant diseases (stroke/CHD/ESRD) and the duration of treatment. Table 2 summarizes the main characteristics of the trials that have already published their results.

CHAOS-2 was the first large randomized trial to be published (in 2002). The investigators studied 1882 CHD patients for a median of 1.7 years, after which the study was terminated early. After a daily dose of 5 mg folic acid, homocysteine levels decreased from 11.2 ± 6.9 mol/L to 9.7 ± 5.3 mol/L; however, there was no reduction in the risk of composite endpoint, which consisted of nonfatal myocardial infarction, cardiovascular death or unplanned revascularization (RR 0.97, 95% CI 0.72–1.29) [67].

The VISP trial enrolled 3680 patients with nondisabling cerebral infarction in 56 centres in the United States, Canada and Scotland between 1997 and 2001; patients were randomized to receive a daily dose of 2.5 mg folic acid, 0.4 mg vitamin B_{12} and 25 mg vitamin B_6 (high-dose arm) or 20 μg, 6 μg and 200 μg, respectively, (low-dose arm), for a mean duration of 2 years. The trial showed that B-vitamin supplementation had no effect on cardiovascular risk and it was terminated prematurely. A possible reason for this negative outcome was that the difference in homocysteine levels between the two groups at the end of the study was only 15% (2 μmol/L), which was lower than the predicted value and was attributed to mandatory fortification of cereals with folic acid after initiation of the trial. Another

Table 2 Large-scale clinical trials examining the effect of B-vitamins on cardiovascular risk.

Trial acronym	Participant (*n*)	Age (years)	Fortified population	Prior disease	Median duration (months)	Folic acid (mg)	Vitamin B_{12} (mg)	Vitamin B_6 (mg)	Reduction in Hcy (%)	RR (95% CI) CHD	RR (95% CI) Stroke	RR (95% CI) All−cause mortality
CHAOS-2	1882	NR	No	CHD	20	5.0	—	—	13.4	1.91 (0.96−3.82)	NR	NR
VISP	3680	66.3	Yes	Stroke	24	2.5	0.4	25	15.7	0.94 (0.73−1.20)	1.04 (0.84−1.29)	0.86 (0.66−1.11)
NORVIT	2815	63.0	No	CHD	36	0.8	0.4	40	29.0	1.08 (0.91−1.29)	0.91 (0.58−1.45)	1.04 (0.82−1.32)
HOPE-2	5522	68.9	Yes	CHD	60	2.5	1.0	50	27.1	0.98 (0.85−1.13)	0.76 (0.59−0.96)	0.99 (0.88−1.11)
HOST	2056	20−56	Yes	ESRD	32	40	2.0	100	25.8	0.86 (0.67−1.08)	0.90 (0.58−1.40)	1.04 (0.91−1.18)
WAFACS	5442	62.8	Yes	CVD	88	2.5	1.0	50	18.5	1.00 (0.85−1.18)	1.14 (0.82−1.57)	0.97 (0.81−1.15)
WENBIT	3096	61.7	No	Suspected CHD	38	0.8	0.4	40	30.0	1.21 (0.95−1.56)	0.72 (0.44−1.17)	1.27 (0.90−1.79)

CVD: cardiovascular disease; CHD: coronary heart disease; ESRD: end-stage renal disease; Hcy: homocysteine; RR: relative risk; NR: not reported.

possible reason could be its modest duration (2 years) [68].

The NORVIT trial was of similar size to VISP but involved patients with recent myocardial infarction in Norway, a non-fortifying country. Patients were randomized to receive one of the following four daily treatments:

- 0.8 mg folic acid, 0.4 mg vitamin B_{12} and 40 mg vitamin B_6;
- 0.8 mg folic acid and 0.4 mg vitamin B_{12};
- 40 mg vitamin B_6;
- or placebo, for a median duration of 40 months.

The study identified a 27% reduction in serum homocysteine levels in patients receiving folic acid plus vitamin B_{12}; however, there was no difference between groups in the primary endpoint (RR 1.08, 95% CI 0.93−1.25, $p < 0.31$), which was a composite of recurrent myocardial infarction, stroke and sudden death attributed to coronary artery disease. Surprisingly, authors reported a trend towards an increased rate of cardiovascular events among patients receiving B-vitamins, in particular the combination of folic acid, vitamin B_6 and vitamin B_{12}. As they commented, the negative results of the trial should not be attributed to reduced compliance or the power of the study. Although slightly underpowered (compared with the original study design), the trial still had an 80% power to detect an 18% reduction in the primary endpoint. The modest duration of the study could be a possible explanation, as well as the fact that mean baseline homocysteine levels were within the normal range. However, as the authors reported, even patients in the upper fifth of baseline homocysteine distribution ($\geq$ 19.7 μmol/L) showed no benefit [69].

The HOPE-2 trial was published simultaneously with the NORVIT trial and reported similar negative results. The study enrolled 5522 patients with a history of vascular disease or diabetes, who were randomized to receive a daily treatment of 2.5 mg folic acid, 50 mg vitamin B_6 and 1 mg vitamin B_{12}, or placebo, for an average of 5 years. Among them, 72.1% of patients originated from countries with mandatory fortification of cereals with folic acid. The study showed no beneficial effect of B-vitamins on primary outcome events and death from cardiovascular causes. However, a significant 24% reduction in stroke incidence (but not transient ischaemic attacks) was demonstrated in the active treatment group (RR 0.75, 95% CI 0.59−0.97) [70].

The HOST trial was the first study to assess the impact of B-vitamin supplementation in patients with advanced chronic kidney disease or ESRD. A total of 2056 participants were enrolled between 2001 and 2006 in 36 centres in the United States for a median follow-up of 32 months. Patients were randomized to receive a daily capsule containing 40 mg folic acid, 100 mg vitamin B_6 and 2 mg vitamin B_{12}, or placebo. Despite a 25.8% reduction in serum homocysteine in the active treatment group, there was no improvement in primary (all-cause mortality) or secondary endpoints, such as incidence of stroke or myocardial infarction. As the investigators suggested, the negative outcome of HOST trial could be attributed to the fact that serum homocysteine reached normal levels in only one-third of patients, despite the administration of the highest vitamin doses among all the homocysteine-lowering studies [71].

WAFACS had the longest follow-up of all the homocysteine-lowering studies as it assessed the effect of B-vitamins (2.5 mg folic acid, 50 mg vitamin B_6 and 1 mg vitamin B_{12}) in 5442 women with a history of CVD or fewer than three cardiovascular risk factors in the United States for a median duration of 7.3 years. Once again, there was no difference between active treatment and placebo groups in terms of myocardial infarction, stroke, coronary revascularization or cardiovascular mortality, despite an 18.5% decrease in homocysteine levels. As with the VISP trial, the implementation of mandatory fortification of cereals with folic acid might have underpowered the study [72].

WENBIT enrolled 3096 patients undergoing coronary angiography in Western Norway, a non-fortifying region. Participants were randomized to one of four groups receiving daily oral treatment with 0.8 mg folic acid, 0.4 mg vitamin B_{12}, 40 mg vitamin B_6; folic acid plus vitamin B_{12}; vitamin B_6 alone; or placebo, for a median duration of 38 months. The study was terminated early after the concerns raised by the NORVIT trial over the safety of the intervention. As with VISP, NORVIT, HOPE-2, HOST and WAFACS, WENBIT did not demonstrate a beneficial effect of B-vitamins on total mortality or cardiovascular events. The trial had less power than originally planned due to lower event rates and shorter follow-up. However, it still had an 80% power to detect a 24% reduction in the risk of composite endpoint comprising all-cause death, nonfatal acute myocardial infarction, acute hospitalization for unstable angina pectoris and nonfatal thromboembolic stroke [73]. A possible explanation for the negative outcome of the study could be that only 9.6% of participants were hyperhomocysteinaemic [74]. As a result, despite a 30% reduction in homocysteine levels in treatment group after 1 year of treatment, serum homocysteine was reduced to a lower value from a higher, but still normal, value [73].

Smaller trials, meta-analyses and observational studies

In addition to the large-scale, randomized trials detailed above, several smaller studies have assessed the role of B-vitamins either directly on cardiovascular risk [75—82] or indirectly on cardiovascular markers such as carotid intima-media thickness (IMT) [82—91]. Wrone et al. studied 510 patients on chronic dialysis allocated to receive 1, 5 or 15 mg folic acid for 2 years and found no difference in mortality and rate of cardiovascular events. Surprisingly, they reported an inverse relationship between homocysteine levels and rate of events [81]. However, another study of 114 dialysis patients reported a positive effect of folate on the cardiovascular event rate [79]. Liem et al. enrolled 593 patients with stable CHD to receive a low dose of folic acid (0.5 mg daily) or placebo for 2 years in a non-fortifying region and they reported that there was no reduction in clinical endpoints [77]. Zoungas et al. used both carotid IMT and clinical events as primary endpoints in 315 patients with chronic renal failure randomized to a high dose of folic acid (15 mg daily) or placebo for a median of 3.6 years, and found no reduction in atheroma progression or cardiovascular morbidity or mortality [82]. In contrast, two recent studies reported a beneficial effect of high-dose B-vitamin supplementation on IMT [85,88].

The meta-analysis by Bazzano et al. in 2006 reported no significant benefit or harm of folic acid supplementation on the risk of CVD, CHD, stroke, or all-cause mortality among patients with a history of CVD or ESRD. As the authors commented, to date, trials have assessed the effect of B-vitamins only in secondary prevention but not in primary prevention [92]. Indeed, the meta-analysis of Wang et al. in 2007 showed that folic acid supplementation can reduce the risk of stroke effectively by 18% in general (RR 0.82, 95% CI 0.68—1.00) and by 25% in primary prevention (RR 0.75, 95% CI 0.62—0.90). Moreover, they showed that a greater beneficial effect was seen in trials with a treatment duration greater than 36 months (RR 0.71, 95% CI 0.57—0.87), a decrease of serum homocysteine greater than 20% (RR 0.77, 95% CI 0.63—0.94) and no fortification or partly fortified grain (RR 0.75, 95% CI 0.62—0.90) [93].

In a population-based cohort study, Yang et al. evaluated trends in stroke-related mortality before and after folic acid fortification in the United States and Canada and, as a comparison, during the same period in England and Wales, where fortification was not implemented. Interestingly, they found that the decrease in stroke mortality observed in the 1990—1997 period in the United States and Canada, accelerated in the 1998—2002 period, with a change from 0.3% to 2.9% per year. On the contrary, stroke mortality did not change significantly between these two periods in England and Wales [94]. As authors suggested, these findings are consistent with the hypothesis that folic acid fortification contributes to the reduction of stroke mortality and overall cardiovascular risk. In this regard, cereal fortification with folic acid would be an additional benefit along with the reduction of neural tube defects. Even if the cardiovascular effect of folic acid fortification is not as powerful as was considered originally, the duration of this intervention (practically throughout life) and its wide application beyond social and financial barriers would contribute significantly towards cardiovascular prevention [74,95].

There are several reasons that may account for the conflicting results of the trials: firstly, we should take into consideration that the study populations were heterogeneous (e.g. nondisabling cerebral infarction in VISP, recent myocardial infarction in NORVIT, diabetes mellitus or vascular disease in HOPE-2). Secondly, the trial durations were short to moderate (20—88 months), especially when B-vitamin supplementation is used as a measure of primary prevention. Moreover, several trials (WENBIT, WAFACS) also recruited normohomocysteinaemic individuals. It should also be noted that trials did not consider patients on an individual basis, and in that sense they may have failed to treat the underlying cause of hyperhomocysteinaemia (e.g. drugs). Finally, folic acid has been shown to induce the remethylation of homocysteine to methionine with increasing SAM (Fig. 1), leading to increasing asymmetrical dimethylarginine levels, which may inhibit endothelial nitric oxide synthase. In addition, enhancing the methylation pathway affects the expression of several proatherogenic genes [96,97].

Conclusion

There is still significant controversy over the 'homocysteine hypothesis' and the possibility that B-vitamin supplementation contributes to the prevention of CVD [98]. Several large-scale clinical trials have reported negative results; possible explanations include trial duration, as well as the impact of folic acid fortification on their power. Currently, more trials are being conducted and their findings are anticipated eagerly. The preplanned meta-analysis of these trials will probably provide strong evidence about the cardiovascular effect of B-vitamins and whether the homocysteine-cardiovascular risk association is causal or just an epiphenomenon [99]. This meta-analysis is designed to involve approximately 52,000 patients, with adequate power to detect a 10% reduction in major cardiovascular events. Moreover, it may identify specific populations (e.g. hyperhomocysteinaemic patients, primary prevention) in which B-vitamins may be beneficial. However, until then, supplementation with B-vitamins for the prevention of CVD is not justified.

Conflicts of interest

None.

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