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The role of vitamins in the pathogenesis and treatment of hyperhomocyst(e)inaemia

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Summary: The relation between vitamin nutritional status and circulating plasma homocyst(e)ine concentrations is reviewed. Several studies have shown that plasma concentrations of folate, vitamin B_{12} and pyridoxal 5′-phosphate are inversely associated with plasma total homocyst(e)ine concentrations. Of the three vitamins mentioned above, folate is the most powerful homocyst(e)ine lowering agent and a daily supplement of 0.65mg/day is sufficient to normalize moderate hyperhomocyst(e)inaemia in most individuals with normal renal function. In patients with severe renal failure, high doses of folate are required to treat hyperhomocyst(e)inaemia. Folic acid is ineffective in reducing plasma total homocyst(e)ine concentrations in patients with a vitamin B_{12} deficiency. Vitamin B_6 supplementation has no effect on fasting plasma total homocyst(e)ine concentrations, but attenuates the post-methionine load plasma homocyst(e)ine peak.

At least one report has shown that some individuals appear to be unable to maintain plasma total homocyst(e)ine concentrations in the normal reference range by a dietary intake of folic acid only. Long-term vitamin supplementation may be indicated in these individuals. However, the clinical benefit of vitamin supplementation has not yet been demonstrated and controlled trials are urgently required.

The sulphur-containing amino acid homocysteine stands at the intersection of two metabolic pathways, i.e. transsulphuration and remethylation (Stipanuk 1986). In the transsulphuration pathway, the condensation of homocysteine with serine to form cystathionine is catalysed by the enzyme cystathionine β-synthase (EC 4.2.1.22). In a subsequent step, cystathionine is hydrolysed by the enzyme γ-cystathionase (EC 4.4.1.1) to yield cysteine and α-ketoglutarate. Both these reactions require the physiologically active form of vitamin B_6, pyridoxal 5′-phosphate (PLP), as essential cofactor. The remethylation of homocysteine is catalysed by *N*-5-methyltetrahydrofolate:homocysteine methyltransferase (EC 2.1.1.13) in a vitamin B_{12}-dependent reaction which transfers the methyl group from *N*-5-methyltetrahydrofolate to homocysteine resulting in the formation of methionine. The methyl group of *N*-5-methyltetrahydrofolate is in fact synthesized *de novo* when a carbon unit is transferred from a suitable source (e.g. serine) to tetrahydrofolate. This reaction produces methylenetetrahydrofolate which is subsequently reduced to *N*-5-methyltetrahydrofolate by the riboflavin-dependent enzyme methylene tetrahydrofolate reductase

(MTHFR; EC 1.1.1.68) (Brody 1991). Alternatively, homocysteine may acquire a methyl group from betaine in a reaction catalysed by betaine:homocysteine methyltransferase (EC 2.1.1.5). Effective cellular homocysteine metabolism is therefore dependent on an adequate status of the essential nutritional factors mentioned above.

The relationship between homocysteine metabolism and cofactor status has been exploited in the treatment of homocyst(e)inuria due to inborn errors of metabolism. Homozygotes for cystathionine β-synthase deficiency have been treated with high-dose pyridoxine supplementation with varying degrees of success (Carson and Carre 1969; Mudd et al 1985), while patients with MTHFR deficiency may benefit from high-dose folate and/or vitamin B_{12} supplementation (Carey et al 1968; Harpey et al 1981). The numerous studies reported during the past decade indicating a possible role for milder forms of hyperhomocyst(e)inemia in premature cardiovascular disease have resulted in a renewed interest in vitamin nutritional status as a determinant of circulating total homocyst(e)ine concentrations (Total homocyst(e)ine refers to the sum of the concentrations of free homocysteine, protein-bound homocysteine, the disulphide homocystine, and the mixed disulphide homocysteine-cysteine.) (Arnesen et al 1995; Israelsson et al 1988; Stampfer et al 1992).

VITAMIN NUTRITIONAL STATUS AS A DETERMINANT OF CIRCULATING TOTAL HOMOCYST(E)INE (tHcy) CONCENTRATIONS

Several studies performed before 1990 indicated that subclinical deficiencies in folate, vitamin B_6 and vitamin B_{12} were associated with hyperhomocyst(e)inaemia. Lindenbaum and colleagues (1988) found that patients with vitamin B_{12} deficiency, but without anaemia or macrocytosis, had markedly elevated total homocyst(e)ine (tHcy) concentrations, while Stabler and colleagues (1988) reported that 77 patients out of 78 with vitamin B_{12} deficiency had serum tHcy concentrations above the normal reference range. Similarly, at least 84% of individuals with subnormal serum folate concentrations also had hyperhomocyst(e)inaemia (Kang et al 1987), while an earlier report suggested that vitamin B_6 depletion resulted in increased urinary tHcy excretion (Park and Linkswiler 1970).

In 1993, Ubbink and colleagues (1993a) reported that the prevalence of suboptimal vitamin B_6, B_{12} and folate status in South African men with hyperhomocyst(e)inaemia was 25.0%, 56.8% and 59.1%, respectively. This observation suggested a strong relationship between poor vitamin nutritional status and elevated circulating tHcy concentrations, a finding which was confirmed when Selhub and coworkers (1993) published the result of their cross-sectional analysis of plasma tHcy concentrations and circulating vitamin concentrations in elderly participants from the Framingham study. These workers found a strong inverse association between plasma tHcy and folate concentrations. The mean plasma tHcy concentrations in subjects in the two lowest deciles of the plasma folate concentration frequency distribution were significantly higher compared to the mean tHcy concentration of individuals in the highest decile of the plasma folate concentration. Plasma tHcy concentrations showed similar inverse relations with plasma vitamin B_{12} and PLP concentrations (Selhub et al 1993). Joosten and coworkers (1993) demonstrated that 14.4% of the variation in plasma tHcy concentrations in elderly people was explained by plasma folate concentrations. In contrast, only 4.4% of the variation in plasma tHcy

concentrations could be explained by the variation in plasma folate concentrations in younger people (Joosten et al 1993). Compared with younger people, the inverse relation between plasma folate and tHcy concentrations was thus more accentuated in elderly people.

The inverse associations between plasma tHcy and vitamin concentrations were not only observed in apparently healthy individuals, but also in different patient groups. Hopkins and colleagues (1995) confirmed the presence of an inverse association between plasma folate and tHcy concentrations as reported previously, but found that patients with early familial cardiovascular disease (CVD) displayed an increased sensitivity with respect to the effects of folate status on plasma tHcy concentrations. A cross-sectional survey of 1041 elderly subjects from the Framingham Heart Study demonstrated that low plasma concentrations of folate and PLP as well as high tHcy levels were associated with an increased risk of extracranial carotid artery stenosis (Selhub et al 1995). Adjustment of plasma vitamin concentrations for tHcy concentrations diminished the strength of the association between vitamin status and carotid artery stenosis prevalence, indicating that the regulatory effect of these vitamins on plasma tHcy concentrations explains why a low vitamin status is linked to an increased risk for carotid artery stenosis (Selhub et al 1995). In a case-control study involving 101 white men with angiographically demonstrated CVD, Pancharuniti and colleagues (1994) also found that the plasma folate concentration was related to CVD risk. After adjustment for plasma tHcy concentrations, the risk was abolished, indicating that a low plasma folate concentration is not an independent CVD risk factor, but that the effect of folate status on CVD is mediated through its effect on plasma tHcy concentrations. Similarly, Dalery and colleagues (1995) found in 150 French-Canadian subjects with angiographically documented CVD a significant inverse relation between plasma folate and tHcy concentrations, again indicating that folate status may influence CVD progression by its effect on plasma tHcy concentrations.

To summarize: Observational studies have shown that vitamin nutritional status is a strong determinant of plasma tHcy concentrations. In particular, a compromised folate status has consistently been linked to hyperhomocyst(e)inaemia. These observations have spurred further research to define the optimum method of vitamin supplementation in order to reduce elevated circulating tHcy concentrations.

THE EFFECT OF VITAMIN SUPPLEMENTATION ON PLASMA tHcy CONCENTRATIONS

Folic acid: Several studies on various population groups have been performed to demonstrate the effect of vitamin supplementation on plasma tHcy concentrations. From the data summarized in Table 1, it is apparent that folate is the most powerful tHcy-lowering agent. Folate has been used in daily doses ranging from 0.65 to 10mg/day, and it seems that in apparently healthy volunteers a low daily dose of 0.65mg or less may be sufficient to maintain plasma tHcy concentrations within the normal reference range (Ubbink et al 1994). This low folate dose may, however, be insufficient in various pathological conditions predisposing towards coronary heart disease. In patients with severe chronic kidney failure, 10mg of folate per day administered for 3 months failed to reduce plasma tHcy concentrations to normal in all the participants (Chauveau et al 1996), while 5mg of

Table 1 A summary of intervention trials to lower circulating, fasting tHcy concentrations

Vitamins and dose[a]	*Supplementation period*	*n*	*Percentage change in circulating tHcy concentration*	*Remarks*
Folate 5 mg	2 weeks	13	−52%, $p<0.01$	Open study on healthy volunteers
Cyanocobalamin 1 mg	2 weeks	14	+7%, NS	
Pyridoxine 40 mg	2 weeks	15	−5%, NS	
Combination: Folate (1.0 mg) Pyridoxine (10 mg) Vitamin B_{12} (0.4 mg)	6 weeks	13	−59.8%, $p<0.001$	Placebo-controlled trial on hyperhomocyst(e)inaemi
Pyridoxine 300 mg	4 months	18	+23.2%, $p<0.05$[b]	Dialysis patients; open trial
Folate	4 months	18	−33.4%, $p=0.001$	
Pyridoxine 10 mg	6 weeks	18	−4.5%, NS	Randomized, placebo-contr
Cyanocobalamin 0.4 mg	6 weeks	17	−14.8%; $p<0.05$	trial on apparently health
Folate 0.65 mg	6 weeks	19	−41.7%, $p<0.001$	individuals with moderat
Combination of above	6 weeks	20	−49.8%, $p<0.001$	hypohomocyst(e)inemia
Pyridoxine 50 mg	6 weeks	10	+8%, NS	Patients had documented ar
Pyridoxine 250 mg	6 weeks	10	−13%, NS	occlusive disease
Pyridoxine 250 mg + Folate 5 mg, combined	6 weeks	10	−53%, $p<0.006$	

Table 1 Continued

Vitamins and dose[a]	*Supplementation period*	*n*	*Percentage change in circulating tHcy concentration*	*Remarks*
Pyridoxine 250 mg Folate 5 mg, combined	6 weeks	72	−51%	Open study on patients wi premature vascular disease
Folic acid, 2.5 or 10 mg	6 weeks	33	−27%, $p<0.001$	Open study on patients wi acute MI
Combination: Folate (1.1 mg) Pyridoxine (5 mg) Vitamin B_{12} (1 mg)	Eight intramuscular injections over 3 weeks	88	−31.3%, $p<0.001$[c]	Placebo-controlled random trial on elderly subjects
Pyridoxine 70 mg	3 months	37	0–18.4%, NS	Nondialyzed patients with renal failure
Folate 10 mg	3 months	37	−33 to −40%, $p<0.001$	Open trial
Cyanocobalamin 2 mg	1 week	235	–4.8%; $p<0.05$	Open study on 126 women
Folate 10 mg	1 week	235	−16.2 to −33.6%, $p<0.0011$[d]	109 men, all apparently he

[a]Unless specified, the daily dose was administered orally
[b]The increase in plasma tHcy concentration is explained by an insufficient washout period prior to the start of the study
[c]Calculated from graph
[d]Two significantly different clusters were observed: individuals with an initial low tHcy concentration ($n=182$) showed a smalle
compared with individuals ($n=53$) with an initial high tHcy concentration
NS = not significant

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