editorial

MILD HYPERHOMOCYSTEINEMIA AND ARTERIAL OCCLUSIVE DISEASE

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The initial link between homocysteine and vascular disease was made by McCully approximately 25 years ago.[1] He observed that an infant who died as a result of a rare genetic condition of abnormal cobalamin metabolism with homocystinuria exhibited widespread, severe arteriosclerosis analogous to the lesions seen in cases of homocystinuria caused by a genetic cystathionine β-synthase deficiency. Because hyperhomocysteinemia was the only condition common to these two metabolic disorders, McCully proposed that hyperhomocysteinemia resulted in arteriosclerotic disease. Although McCully's hypothesis did not gain immediate support, the association between plasma homocysteine concentration and arteriosclerosis has more recently become the subject of a number of clinical studies. In 1976 Wilcken and Wilcken showed that the concentration of homocysteine-cysteine mixed disulphide after a methionine load was slightly higher in CHD patients than in respective age-sex matched controls.[2] This pioneering work has led to many studies which have been the subject of two important review articles. The first by Ueland *et al*.[3] summarized 17 studies that presented fasting homocysteine concentrations for approximately 1500 patients with various forms of vascular disease and a similar number of normal controls. Fasting homocysteine concentrations were consistently elevated among patients with all types of vascular disease and averaged 31% greater than concentrations among controls. Abnormal homocysteine metabolism was also measured in some studies by presenting individuals with a large oral dose of methionine, which is a homocysteine precursor. Frequencies of abnormal homocysteine response to methionine loading were higher (24%) in patients with vascular disease than in healthy controls (2%). The more recent metanalysis by Boushey *et al*.[4] of 27 studies including prospective and population-based case-control studies concluded that elevations of total plasma homocysteine (tHcy) were considered an independent graded risk factor for arteriosclerotic vascular disease, with odds ratios for a 5 umol/L increase in plasma tHcy that were equal to 1.5-1.8 for men and women with CHD, cerebrovascular or peripheral vascular diseases.

Our interest in homocysteine was prompted by the possibility that plasma homocysteine may serve as an indicator of the status and perhaps the intake of a number of vitamins, including folic acid, vitamin B_{12} and vitamin B_6. This possibility derived from the large number of studies which implied that methionine metabolism is tightly regulated,[3,6,7] and from other studies which showed that deficiencies in the above vitamins are often associated with hyperhomocysteinemia.[8-18]

The aims of our studies were therefore: 1) to determine the relationship between plasma homocysteine concentrations and status and intake of folate, vitamin B_{12} and vitamin B_6; 2) to determine the prevalence of hyperhomocysteinemia; 3) to determine the contribution of vitamin status and intake to the prevalence of hyperhomocysteinemia and, 4) to describe the associations between plasma homocysteine, vitamin status and intake and prevalence of carotid artery stenosis.

In our recent studies[19,20] we examined members of the original Framingham Heart Study cohort, a population-based sample of 5209 men and women originally examined in 1948-1952[21] and followed prospectively to the present to assess the occurrence of vascular disease. This study was based on 1401 survivors of the original cohort who participated in the 20th biennial examination (1989-90). Homocysteine and carotid ultrasound measures were available for 1041 individuals (418 men and 623 women), aged 67 to 96 years old at the time of data collection. The main findings of these studies were the following.

Homocysteine distribution and prevalence of high homocysteine concentrations

The mean homocysteine concentration for all subjects was 11.9 μmol/L (median = 11.6 μmol/L).

Values ranged from 3.5 to 66.9 µmol/L. Homocysteine concentration was higher in men than in women and increased with age. The increase with age remained highly significant (p < 0.001) for men and women after adjustment for plasma vitamin concentrations, but the difference between men and women was no longer statistically significant. We defined high homocysteine as concentrations greater than the 90th percentile among subjects with all plasma vitamin levels >70th percentile (14.0 µmol/L). Prevalence of high homocysteine was 29.3% for the entire cohort and over 40% for individuals aged 80 years and older.

Mean homocysteine concentration by vitamin status and intake

Folate. Mean plasma homocysteine concentrations for subjects in the two lowest deciles of plasma folate (below 4.8 nmol/L) were 15.6 and 13.7 µmol/L. These were significantly greater than the mean for subjects in the highest decile, which was 11.0 µmol/L (p<0.01). Mean homocysteine concentrations for subjects in the three lowest deciles of folate intake (less than 253 µg/day) were 13.7, 12.9 and 13.2 µmol/L, respectively, and were significantly greater than the mean for subjects in the highest intake decile, which was 10.4 µmol/L (p < 0.01).

Vitamin B_{12}. Mean homocysteine concentrations were significantly elevated for subjects in the lowest decile for vitamin B_{12} relative to subjects in the highest decile (p < 0.01). Mean homocysteine concentrations were 15.4 and 10.9 µmol/L for subjects in the lowest and highest vitamin B_{12} deciles. Subjects in the lowest vitamin B_{12} decile had vitamin B_{12} concentrations below 139 pmol/L. Vitamin B_{12} intake appeared to be unrelated to mean homocysteine concentration, even though subjects in the fifth decile had significantly higher homocysteine concentrations than subjects in the highest decile (p < 0.05).

Vitamin B_6. Mean homocysteine concentrations were significantly elevated for subjects in the lowest decile for PLP relative to subjects in the highest decile for this vitamin (p<0.01). Mean homocysteine concentrations were 14.3 and 10.9 µmol/L for subjects in the lowest and highest PLP deciles. Subjects in the lowest decile had PLP concentrations below 18.1 nmol/L. For vitamin B_6 intake, mean homocysteine concentrations were significantly elevated in the lowest two deciles (p<0.01) and the third decile (p<0.05). Mean homocysteine concentrations were 13.4, 12.4 and 12.3 µmol/L for subjects in the lowest three deciles; the mean in the highest decile was 10.1 µmol/L. Subjects in the lowest three intake deciles reported consuming less than 1.75 mg/day.

Homocysteine concentrations by overall vitamin status

Mean homocysteine and the prevalence of high homocysteine increased dramatically across cate-

gories of the B vitamin index (Table 1). Mean homocysteine concentration was 75% greater in the lowest relative to the highest index categories. The prevalence of high homocysteine was almost 6-fold greater among subjects in the lowest index category compared with subjects in the highest category for plasma index. Sixty-seven percent of the cases of high homocysteine in this cohort of older subjects were associated with at least one vitamin concentration below the 70th percentile. Although the prevalence of high homocysteine was substantially greater in lower vitamin categories (4 and 5) than in the middle category, this latter category contributed the largest share of cases of high homocysteine for the index because it included the largest proportion of the cohort.

Relationship between plasma homocysteine and prevalence of extracranial stenosis

The prevalence of extracranial carotid stenosis ≥ 25 percent was approximately 43 percent and 34 percent in men and women, respectively. In men, the prevalence of stenosis ≥ 25 percent was 27 percent (95 percent confidence interval: 17 to 38 percent) in the lowest homocysteine quartile and 58 percent (95 percent confidence interval: 49 to 67 percent) in the highest quartiles ($p_{trend} < 0.001$). The relationship in women was not as striking as that in men; prevalence of stenosis ≥ 25 percent ranged from 31 percent (95 percent confidence interval: 24 to 38 percent) to 39 percent (95 percent confidence interval: 31 to 47 percent) across homocysteine quartiles (p_{trend}=0.03). While the risk of stenosis appeared to increase in the second homocysteine quartile (9.1 to 11.3 µmol/L) among men, it did not appear to increase until the third homocysteine quartile (11.4 to 14.3 µmol/L) among women. Although the prevalence of stenosis appeared somewhat greater among men than women in the upper quartiles of homocysteine, a test of interaction between sex and homocysteine

Table 1. Elevated homocysteine concentrations by B vitamin status.

B vitamin index		n.	Mean homocysteine (µmol/L)	Prevalence (%)	Prevalence rate ratio	Attributable percent	Population attributable percent
Highest	1	89	9.4	10.1	1	0.0	0.0
	2	128	9.8	12.5	1.2	19.2	1.0
	3	534	11.9*	28.7*	2.8	64.8	33.7
	4	144	14.9*	52.1*	5.2	80.6	20.6
Lowest	5	70	16.5*	58.6*	5.8	52.8	11.6
					(Total	66.9)	

1 = index combines plasma folate, vitamin B_{12} and PLP concentration; high 1) = all three B vitamins > 70th percentile; 2 = all vitamins > 50th, at least 1 < 70th percentile; 3 = vitamins above and below the 50th percentile; 4 = all vitamins < 50th percentile, at least 1 > 30th percentile; low (5) = all three vitamins < 30th percentile. *Significantly different from category 1 (p<0.01).

Table 2. Odds ratios of maximal extracranial carotid artery stenosis > 25 percent according to quartile of plasma vitamin concentrations.

	N	Odds Ratio	95% CI*	p value	Odds Ratio	95% CI*	p value
		Adjusted for multiple risk factors			*Adjusted for multiple risk factors plus homocysteine*		
Folate (ug/L)							
<2.51	1,027	1.9	1.3,2.7	0.001	1.5	1.0,2.3	0.04
2.51-4.31		1.4	1.0,2.0	0.08	1.3	0.9,1.9	0.24
4.32-7.92		1.2	0.8,1.8	0.28	1.2	0.8,1.8	0.35
7.93		1.0			1.0		
P_{trend}				<0.001			0.05
Vitamin B_{12} (ng/L)							
<290	881	1.4	0.9,2.1	0.11	1.2	0.8,1.8	0.41
290-405		1.4	0.9,2.0	0.14	1.2	0.8,1.8	0.36
406-572		1.3	0.9,2.0	0.16	1.3	0.9,1.9	0.24
573		1.0			1.0		
P_{trend}				0.11			0.47
Vitamin B_6 (nmol/L)							
<31.61	967	1.6	1.1,2.4	0.02	1.3	0.9,2.0	0.15
31.91-52.19		1.1	0.7,1.6	0.67	1.0	0.6,1.4	0.80
52.20-89.80		1.2	0.8,1.7	0.48	1.1	0.7,1.6	0.71
89.81		1.0			1.0		
P_{trend}				0.03			0.2

*CI: confidence interval.

indicated that the trends for prevalence of stenosis ≥ 25 percent were not significantly different for men and women (p=0.07).

The associations between carotid stenosis and plasma vitamins are shown in Table 2. The prevalence of stenosis ≥ 25 percent was inversely associated with both folate (p_{trend} < 0.001) and pyridoxal-5'-phosphate (p_{trend} = 0.03) after adjustment for age, sex and other risk factors. The odds ratio for stenosis was 1.9 (95 percent confidence interval: 1.3 to 2.7) in the lowest folate quartile and 1.6 (95 percent confidence interval: 1.1 to 2.4) in the lowest pyridoxal-5'-phosphate quartile. Plasma vitamin B_{12} exhibited a weak association with stenosis (P_{trend} = 0.11). The odds ratio for stenosis was 1.4 (95 percent confidence interval: 0.9 to 2.1) in the lowest vitamin B_{12} quartile compare with the highest quartile. Adjustment for homocysteine diminished the strength of plasma vitamin associations, but the elevated prevalence of stenosis in the lowest plasma folate quartile remained evident (odds ratio: 1.5; 95 percent confidence interval: 1.0 to 2.3).

These data suggest an important role for nutritional status in homocysteine metabolism. We have demonstrated strong, non-linear, inverse associations between homocysteine concentrations and plasma concentrations of folate, vitamin B_{12}, and vitamin B_6. We observed that individuals with low levels of each of these vitamins had high plasma homocysteine concentrations, while those with moderate vitamin levels had dramatically lower homocysteine concentrations. Homocysteine levels did not differ substantially between individuals with moderate and high vitamin concentrations.

The results for folate and vitamin B_6 intake data

are consistent with those for the plasma vitamins. Although it is risky to attribute discrete quantitative values based on this method of dietary assessment,[22] it still may be worth noting that homocysteine concentrations were elevated among individuals with folate intakes up to 280 µg/day, which is higher than the current RDA of 200 and 180 µg/day for adult men and women, and vitamin B6 intakes as high as 1.92 mg/day, which is less than the RDA of 2.0 mg/day for men but greater than the RDA of 1.6 mg/day for women.

Adequate levels of all three vitamins may be needed to obtain an optimal homocysteine concentration. Using the index based on levels of all three vitamins, we estimated that approximately two-thirds of the cases of elevated homocysteine concentration in this cohort were associated with low or moderate plasma levels of one or more of the three vitamins.

Our findings also provide evidence that plasma homocysteine levels are associated with extracranial carotid stenosis in a population-based, elderly cohort. We observed that risk of stenosis ≥ 25 percent was increased at homocysteine concentrations previously believed to be normal based on levels of homocysteine among normative samples. As in our previous analysis, we defined elevated plasma homocysteine as concentrations greater than 14 µmol/L (90th percentile among individuals with apparently adequate folate, vitamin B_{12} and vitamin B_6 status). Stampfer et al.[23] defined elevated homocysteine as concentrations greater than 15.8 µmol/L (95th percentile among non-diseased control subjects). Joosten et al.[24] defined elevated homocysteine as concentrations greater than 13.9 µmol/L (mean plus 2 standard deviations among healthy young controls). Genest and coworkers[25] reported 90th and 95th percentile values of 15.0 and 19.0 µmol/L among their normal controls. In the present study we observed that risk of stenosis was elevated at levels of homocysteine between 11.4 and 14.3 µmol/L. These data will require us to reconsider the current beliefs regarding standards for elevated homocysteine.

We also examined the relationships between specific nutritional determinants of hyperhomocysteinemia and stenosis in this elderly cohort. We further demonstrated that folate and pyridoxal-5'-phosphate were linked to stenosis, in large part, due to their regulation of plasma homocysteine levels as indicated by the diminished odds ratios between stenosis and these vitamins after adjustment for homocysteine levels. Although there was some residual association between plasma folate and stenosis after adjustment for homocysteine, the likelihood ratio test statistic would suggest that addition of folate to a model containing homocysteine did not add any significant contribution. It is likely that measurement error and biological variability in both folate and homocysteine might explain the

residual folate association.

We demonstrated that the majority of these elderly individuals with elevated homocysteine concentrations have insufficient folate, vitamin B_{12} or vitamin B_6 status, and others have shown that innocuous vitamin supplementation regimens (including folate, B_{12}, and B_6) effectively lower moderately elevated plasma homocysteine levels to the normal range.[10,18,26-33]

Results of our studies provide the rationale for a randomized, controlled trial of the effect of homocysteine-lowering vitamin therapy on vascular disease morbidity and mortality in hyperhomocysteinemic, elderly individuals. The relevance of this topic was emphasized at the *Meeting on Basic, Laboratory and Clinical Aspects of Thromboembolic Diseases,* held in La Thuile, Italy, on March 17-23, 1996, and two papers presented on that occasion are reported in this issue of Haematologica. While the first winter meeting held in 1994 was mainly devoted to genetic disorders responsible for venous thrombosis,[32-36] the 1996 one highlighted the relevance of acquired disorders as a cause of arterial thrombosis.

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