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What level of plasma homocyst(e)ine should be treated? Effects of vitamin therapy on progression of carotid atherosclerosis in patients with homocyst(e)ine levels above and below 14 μmol/L

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What Level of Plasma Homocyst(e)ine Should Be Treated?

Effects of Vitamin Therapy on Progression of Carotid Atherosclerosis in Patients With Homocyst(e)ine Levels Above and Below 14 μmol/L

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High levels of plasma homocyst(e)ine (H[e]) are associated with increased vascular risk. Treatment is being contemplated, but the level at which patients should be treated is not known. We compared the response of carotid plaque to vitamin therapy in patients with H(e) above and below 14 μmol/L, a level commonly regarded as high enough to warrant treatment. Two-dimensional B-mode ultrasound measurement of carotid plaque was used to assess the response to vitamin therapy with folic acid 2.5 mg, pyridoxine 25 mg, and cyanocobalamin 250 μg daily, in 101 patients with vascular disease (51 with initial plasma levels above, and 50 below 14 μmol/L). Among patients with plasma H(e) >14 μmol/L, the rate of progression of plaque area was 0.21 ± 0.41 cm^2/year before vitamin therapy, and −0.049 ± 0.24 cm^2/year after vitamin therapy ($P2 = .0001$; paired *t* test). Among patients with levels <14 μmol/L, the rate of progression of plaque was 0.13 ± 0.24 cm^2/year before vitamin therapy and −0.024 ± 0.29 cm^2/year after vitamin therapy ($P2 = .022$, paired *t* test). The change in rate of progression was −0.15 ± .44 cm^2/year below 14 μmol/L, and −0.265 ± 0.46 cm^2/year above 14 μmol/L ($P = 0.20$). Vitamin therapy regresses carotid plaque in patients with H(e) levels both above and below 14 μmol/L. These observations support a causal relationship between homocyst(e)ine and atherosclerosis and, taken with epidemiologic evidence, suggest that in patients with vascular disease, the level to treat may be <9 μmol/L. Am J Hypertens 2000; 13:105–110 © 2000 American Journal of Hypertension, Ltd.

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Since the discovery of homocystinuria in 1962,[1,2] it has become clear that hyperhomocyst(e)inemia is a strong independent risk factor for vascular disease.[3–6] Vitamin therapy reduces plasma homocyst(e)ine levels, and reverses endothelial dysfunction associated with hyperhomocyst(e)inemia.[7–9] Whether vitamin therapy can prevent cardiovascular events is currently under investigation. An important unresolved issue is the level of plasma homocyst(e)ine at which treatment should be considered.

Homocyst(e)ine [H(e)] plasma levels >14 μmol/L are generally considered high; but this level, based on the "normal distribution," may be too high to be regarded as a level below which treatment would not be indicated. Plasma H(e) > 10.2 μmol/L is associated with a doubling of vascular risk,[5] and the slope of the H(e)/risk relationship is steep. The relative risk of coronary events is 9.9 times higher for H(e) > 20 versus < 9 μmol/L,[6] suggesting that treatment may reduce risk.

Although, in the past, physicians often focused on treatment of patients with high blood levels of cholesterol or its fractions such as LDL, more recently, consensus guidelines focus on identifying high risk patients, and treating patients with vascular disease to low target levels such as <2.6 mmol/L of LDL.[10] We believe that a similar construct will apply to the treatment of H(e), once it is demonstrated, that treatment prevents events.

In our Atherosclerosis Prevention Clinic, we routinely determine whether therapy is successful by following progression of two-dimensional carotid plaque area:[11] a multiple regression model, in which Framingham risk factors predict carotid plaque area, is used to identify patients with Unexplained Atherosclerosis, who are likely to have new genetic causes.[12] Among patients with Premature Atherosclerosis identified clinically, 33% had homocyst(e)ine levels >14 μmol/L, compared with 20.1% of volunteers; among the top 10% of cases (ie, those with residual scores in the top 10% in the regression model), 60% had plasma H(e) >12 μmol/L, and 14% have both a plasma H(e) >14 μmol/L and a plasma Lp(a) level >20 g/L.[12]

In patients in whom plaque is progressing despite control of routine risk factors, including an LDL <2.5 mmol/L, we suspect and treat newer risk factors such as hyperhomocyst(e)inemia and high levels of Lp(a).

A previous letter to the editor presented our findings on rate of progression of carotid atherosclerosis before and after vitamin therapy with folic acid, vitamin B6, and B12 in 38 patients with H(e) >14 μmol/L.[13] We report here a comparison of the response to vitamin therapy of patients with levels above and below 14 μmol/L.

METHODS

Patient Population The patients were all attending either the Atherosclerosis Prevention Clinic or the Premature Atherosclerosis Clinic of the London Health Sciences Centre in London, Ontario, Canada. The patients had been referred because of multiple risk factors (in most cases, with atherosclerotic vascular disease), or because they had vascular disease without the usual risk factors. All patients in whom valid carotid plaque area measurements were available before and after treatment with vitamin therapy were included. (Occlusion of the artery invalidates the plaque measurements, as it is not possible to distinguish between plaque and organized thrombus.) There were 51 cases with plasma H(e) >14 μmol/L, and 50 below that level.

Plasma Homocyst(e)ine Levels Before 1995, plasma homocyst(e)ine was measured by HPLC at the Oregon Primate Research Center laboratory (Dr. Rene Malinow).[14] Since then, H(e) assays have been done at the Robarts Research Institute, London, Ontario, by Dr David Freeman, using the method of Jacobsen et al.[15] The assay was standardized against that of the Oregon Primate Research Center laboratory (Dr. Rene Malinow)[14] and that of Jacobsen at the Cleveland Clinic[15] by doing duplicate assays against each of those laboratories; agreement was within 5% for all samples. The coefficient of variation was <7% throughout the range of levels observed, including high levels equivalent to those seen in dialysis patients.

Treatment All patients were being treated actively with multiple risk factor intervention: diet, exercise, smoking cessation, drug treatment for hyperlipidemia (including combination therapy with fibrates or niacin, and statins for cases with low HDL or high triglycerides); patients who exhibited rapid progression, or whose arteries were not responding to therapy for the usual risk factors, were tested for Lp(a) and homocyst(e)ine.

Vitamin therapy for homocyst(e)ine consisted at first of folic acid 5 mg daily. Since 1996, the treatment regimen was modified to folic acid 2.5 mg with pyridoxine (vitamin B_6) 25 mg and cyanocobalamin (vitamin B_{12}), 250 μg, all given daily.

To evaluate the effect of vitamin treatment on rate of progression of carotid atherosclerosis, we analyzed the rate of progression of carotid plaque before and after treatment with these vitamins. Rate of progression of total plaque area was determined by two-dimensional ultrasound.[16] The rate of change of atherosclerosis for a given patient was taken to be the change in total aggregate plaque area (cm^2) in both common, internal and external carotid arteries di-

TABLE 1. BASELINE CHARACTERISTICS OF THE PATIENTS

	H(e) >14 N = 51	H(e) ≤14 N = 50	Significance*
Age (years; mean ± SD)	53.58 ± 11.24	52.4 ± 9.2	.554
Sex (% female)	49% female	52% female	.919
Total cholesterol (mmol/L)	6.39 ± 1.27	5.99 ± 1.35	.083
Triglycerides (mmol/L)	2.70 ± 2.03	2.50 ± 1.35	.546
HDL (mmol/L)	1.68 ± 2.51	1.32 ± .46	.326
Plasma homocyst(e)ine (μmol/L)	22.45 ± 11.27	10.98 ± 2.12	.0001
Systolic pressure (mm Hg)	142.41 ± 17.02	154.42 ± 124.72	.842
Diastolic pressure (mm Hg)	79.98 ± 12.91	80.44 ± 9.92	.107
Pack-years of smoking	11.53 ± 16.6	8.83 ± 12.55	.360
On treatment with lipid meds (%)	55.8%	38%	.032
On treatment with BP meds (%)	77%	18.4%	.547
History of diabetes (%)	7.8%	4%	.416

t *Test for continuous variables, Mann-Whitney test for categorical variables.*

Values for blood pressure and lipids were the highest values on record for each case.

Meds, medications.

vided by time; rates before (prerate) and after (postrate) the initiation of treatment were calculated.

RESULTS

Patients With Plasma H(e) >14 μmol/L There were 51 patients with levels >14 μmol/L available for analysis. Plasma H(e) ranged from 14.28 to 69.38 μmol/L (mean + SD, 22.45 ± 11.27). Twenty-five (49%) were women, the mean age was 53.58 ± 11.2 years; four patients were diabetic. Table 1 gives the baseline characteristics of the two groups of patients. The mean follow-up of these cases was 4.45 ± 1.52 years.

The mean duration of treatment before initiation of vitamin therapy was 2.6 ± 1.4 years (range 0.9 to 6 years); the posttreatment period was 1.8 ± 0.7 years (0.8 to 3.3 years). Before initiation of vitamin therapy, the rate of atherosclerosis progression was steeply upward, with a rate of progression of 0.21 ± .41 cm^2/year before vitamin therapy, and −0.049 ± 0.24 cm^2/year after vitamin therapy ($P2 = .0001$; paired t test).

Patients With Plasma H(e) ≤14 There were 50 patients with H(e) values ≤ 14 μmol/L, who had measurement of carotid plaque area before and after commencing treatment with folic acid 2.5 mg, pyridoxine 25 mg, and vitamin B_{12} 250 μg daily. The plasma H(e) ranged from 4.7 to 13.93 μmol/L (mean ± SD, 10.98 ± 2.12). There were 26 women. The mean age was 52.4 ± 9.2 years. Two patients were diabetic. Baseline characteristics are given in Table 1.

The duration of follow-up before treatment was 2.7 ± 1.7 years (range, 0.9 to 6.0 years). Follow-up after treatment was 1.6 ± 0.9 years (range, 0.8 to 4.2 years). Before initiation of vitamin therapy, the rate of atherosclerosis progression was steeply upward, with a rate of progression of 0.13 ± 0.24 cm^2/year before vitamin therapy and −0.024 ± 0.29 cm^2/year after vitamin therapy ($P^2 = .022$, paired t test).

Comparison of Responses Above and Below 14 μmol/L As can be seen from the figures, both groups responded to vitamins, with a rapid rate of progression before treatment and a slight regression after treatment: ie, the rate of regression was greater than the rate of progression. There was no significant difference in rate of progression between the two groups; the change in rate of progression was −0.15 + 0.45 cm^2/year below 14 μmol/L, and −0.27 + 0.46 cm^2/year above 14 μmol/L ($P = .203$).

Response to Vitamin Therapy by Age and Sex Men and women were similar in age (men 51.05 ± 10.1 years, women 55.0 ± 10.1 years; $P = .052$) homocyst(e)ine levels (men 18.12 ± 12.11 μmol/L; women 15.35 ± 6.78 μmol/L; $P = .162$) and in rate of plaque progression before vitamin therapy (men 0.197 ± 0.33 cm^2/year, women 0.143 ± 0.36 cm^2/year). Men and women responded equally well to vitamin therapy: the change in rate of progression for men was −0.23 ± 0.50 cm^2/year, versus −0.187 ± 0.40 cm^2/year for women ($P = .67$).

For the 13 patients aged ≥65 years, the rate of progression was significantly higher; the rate for those ≥65 yr was 0.35 ± 0.56 cm^2/year, versus 0.15 ± 0.29 cm^2/year for those <65 yr ($P = .047$). Older patients responded significantly better to vitamin therapy than did younger patients, with a regression rate of −0.48 ± 0.51 cm^2/year for those ≥65 versus −0.17 ± 0.44 cm^2/yr <65 yr ($P = .02$).

Relation to Lipid Lowering Therapy At baseline, 39.6% of the patients were already taking lipid lowering therapy. Partial correlation for age and change in

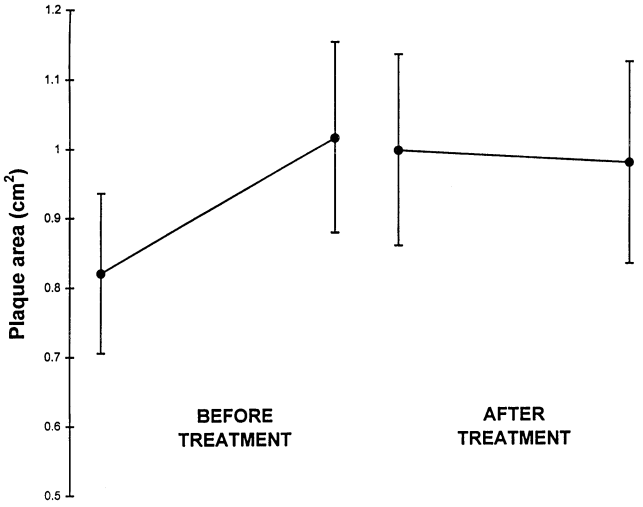

FIGURE 1. *Rate of atherosclerosis progression before and after vitamin treatment in patients with H(e) >14 μmol/L. The mean duration of treatment before initiation of vitamin therapy was 2.6 ± 1.4 years (range 0.9 to 6 years); the post-treatment period was 1.8 ± 0.7 years (range 0.8 to 3.3 years). Before initiation of vitamin therapy, the rate of atherosclerosis progression was steeply upward, with a rate of progression of 0.21 ± 0.41 cm²/year before vitamin therapy, and the rate became negative (ie, regression was observed) at −0.049 ± 0.24 cm²/year after vitamin therapy (*P2 = *0.0001; paired* t *test).*

rate of progression, adjusted for lipid lowering therapy, was 0.313 ($P = .002$). The partial correlation for lipid therapy and change in rate of progression, controlling for age, was −0.1913 ($P = .057$). Stepwise multiple regression was used to identify the strongest model. With age, sex, lipid lowering therapy, and pack-years of smoking as independent variables, and change in rate of progression as the dependent variable, the adjusted R^2 was only 0.119, and only age was a significant predictor in that model.

The issue of hyperlipidemia as a determinant of rate of progression or response to treatment was further analyzed by comparing patients above and below a median split for the highest total cholesterol on record (at 5.97 mmol/L). The rate of progression before treatment was 0.12 ± 0.31 for the lower cholesterol group versus 0.21 ± 0.37 for the high cholesterol group ($P = .84$). The rate of progression after treatment with vitamins was −0.028 ± 0.25 cm²/year for the low cholesterol group, versus −0.046 ± 0.29 cm²/year for the high cholesterol group ($P = .23$); the change in rate from pretreatment to posttreatment was −0.15 ± 0.41 for low cholesterol patients, versus −0.26 ± 0.49 cm²/year for high cholesterol patients ($P = .29$).

DISCUSSION

Despite the strong association between elevated levels of plasma homocyst(e)ine and vascular risk, there is controversy regarding the causality of the association.[17,18] However, with increasing understanding of the biology of hyperhomocyst(e)inemia, a causal relationship is increasingly plausible.

The mechanisms by which elevated levels of homocyst(e)ine aggravate atherosclerosis appear to include increased production of hydrogen peroxide,[19,20] oxidative stress, and endothelial dysfunction,[7–9,21,22] with increased oxidation of LDL and changes in Lp(a),[23] and hypercoagulability.[24,25] Vitamin therapy restores endothelial dysfunction.[8,9,22] Patients with chronic renal failure on hemodialysis have marked increases in vascular risk, of 17-, 19-fold, associated with very high plasma levels of homocyst(e)ine.[26–28] In our dialysis population, the mean level is 28 μmol/L, and there was no difference in plasma homocyst(e)ine levels between patients taking 1 mg versus 5 mg daily of folic acid.[29] Vitamin therapy in hemodialysis patients reduces plasma homocyst(e)ine by approximately 30%, and restores levels of Lp(a) and fibrinogen toward normal.[30] The fact that vitamin therapy reduces very high levels of homocyst(e)ine by only 30% in dialysis patients, leaving levels that are still very high (approximately 20 μmol/L in most cases), suggests that other approaches are required to reduce risk levels in these very high risk patients.[29]

Some readers might wonder why patients with low levels of H(e) between 4.7 and 14 μmol/L were treated at all. This happened because these patients exhibited

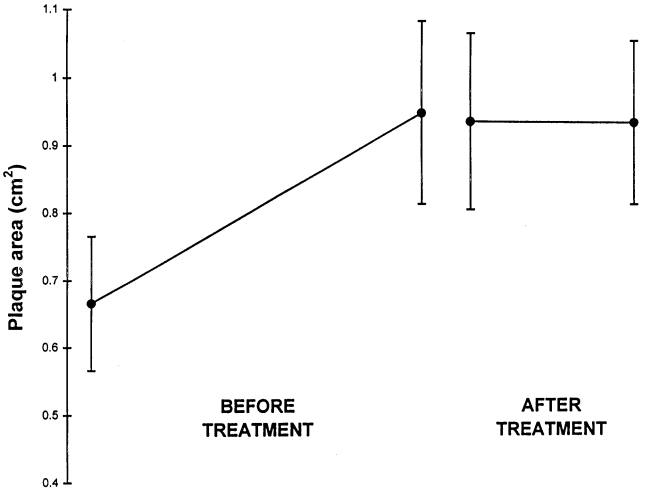

FIGURE 2. *Rate of atherosclerosis progression before and after vitamin treatment in patients with H(e) <14 μmol/L. The duration of followup before treatment was 2.7 ± 1.7 years (range 0.9 to 6.0 years); follow-up after treatment was 1.6 ± 0.9 years (range 0.8 to 4.2 years). Before initiation of vitamin therapy, the rate of atherosclerosis progression was steeply upward, with a rate of progression of 0.13 ± .24 cm²/year before vitamin therapy, and the rate became negative (ie, regression was observed) at −0.024 ± 0.29 cm²/year after vitamin therapy (*P2 = *0.022, paired* t *test).*

rapid progression of their carotid plaque area, despite aggressive treatment of other risk factors, and because most clinic visits are 1 year apart and it takes some time for plasma H(e) levels to be reported by our laboratory. Often the patient was on therapy and showing improvement before the blood level was reviewed 1 year later, and therapy was continued because of the improvement. The levels were only measured while fasting, and the possibility of pyridoxine-responsive cases that might have been revealed by methionine loading was another reason for initiating and continuing therapy when improvement was observed.

Two important weaknesses of this report require discussion: 1) the patients were not randomized to therapy, and 2) posttreatment homocyst(e)ine levels were not available in most cases. Plasma H(e) is not reimbursed by the Ontario health plan, and our hospital is in a deficit position; the reason for not repeating the plasma H(e) was to avoid further increasing the hospital deficit. The patients were all treated because they did not qualify for the clinical trial that we are now conducting in collaboration with others, and because the doses of vitamins used are inexpensive and harmless. Given that the patients were progressing rapidly, despite treatment of all their known risk factors, it therefore seemed reasonable to treat the patients while awaiting the outcome of more definitive studies.

Some have questioned whether the regression may have been due to a delayed effect of lipid lowering therapy rather than to vitamin therapy. The analysis presented in the Results section suggests that, although there may have been an interaction with lipid lowering, a major part of the regression was not due to lipid lowering therapy. Furthermore, the patients were on active treatment of other risk factors for an average of >2 years before vitamin therapy was started, so the effects of lipid lowering would have been expected to occur by then.

The observations reported here support the causal hypothesis, in that vitamin therapy was associated with regression of carotid plaque. It is of great interest that regression was observed even in patients with levels below those conventionally thought to be high enough to warrant therapy. It should be noted, however, that these were patients with unusually rapid progression of disease: for both groups of patients, above and below 14 μmol/L, the rate of progression before treatment was higher than that seen in the patients between the 20th and 80th percentiles of plaque area at baseline, whose average rate of progression was 0.11 cm^2/year.[11]

Clinical trials are underway that will determine the value of vitamin therapy in secondary prevention of events. In the Vitamin Intervention for Stroke Prevention (VISP) trial, a National Institutes of Health trial of secondary prevention in patients with nondisabling stroke, treatment is being initiated at levels above the 25th percentile (ie, >9.5 μmol/L in men, and >8.5 μmol/L in women).

Conclusions In a clinical practice setting, treatment with folic acid, vitamin B_6 and B_{12} halted the progression of carotid plaque in patients with plasma homocyst(e)ine levels above and below 14 μmol/L. The response of women was equal to that of men, and the response of patients with plasma H(e) <14 μmol/L was not significantly less than the response in patients with higher levels. In both groups, the change in rate of progression was >100% (ie, both groups changed from progression to regression). Elderly patients responded better than did younger patients. Taken with epidemiologic evidence, our findings suggest that, in patients with vascular disease, the target level for plasma homocyst(e)ine should be <9 μmol/L. This observation requires confirmation in randomized trials, which are underway. Carotid plaque measured by ultrasound appears to be a very sensitive way to measure the effects of antiatherosclerotic interventions.

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