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HYPERHOMOCYSTEINEMIA AS A RISK FACTOR FOR DEEP-VEIN THROMBOSIS

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Abstract *Background.* Previous studies have suggested that hyperhomocysteinemia may be a risk factor for venous thrombosis. To assess the risk of venous thrombosis associated with hyperhomocysteinemia, we studied plasma homocysteine levels in patients with a first episode of deep-vein thrombosis and in normal control subjects.

Methods. We measured plasma homocysteine levels in 269 patients with a first, objectively diagnosed episode of deep-vein thrombosis and in 269 healthy controls matched to the patients according to age and sex. Hyperhomocysteinemia was defined as a plasma homocysteine level above the 95th percentile in the control group (18.5 μmol per liter).

Results. Of the 269 patients, 28 (10 percent) had plasma homocysteine levels above the 95th percentile for the controls, as compared with 13 of the controls (matched odds ratio, 2.5; 95 percent confidence interval, 1.2 to 5.2). The association between elevated homocysteine levels and venous thrombosis was stronger among women than among men and increased with age. The exclusion of subjects with other established risk factors for thrombosis (e.g., a deficiency of protein C, protein S, or antithrombin; resistance to activated protein C; pregnancy or recent childbirth; or oral-contraceptive use) did not materially affect the risk estimates.

Conclusions. High plasma homocysteine levels are a risk factor for deep-vein thrombosis in the general population. (N Engl J Med 1996;334:759-62.)

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MILD hyperhomocysteinemia is an established risk factor for atherosclerosis and vascular disease.[1,2] In classic homocystinuria, half the vascular complications are of venous origin,[3] but until recently it has been unclear whether mild hyperhomocysteinemia is also a risk factor for venous thrombosis.[2,4,5] In a case–control study, Falcon et al. found that hyperhomocysteinemia was a risk factor for thrombosis in people younger than 40 years of age.[6] They reported that the difference in homocysteine levels between case patients and control subjects was particularly evident after methionine loading (since methionine is a precursor of homocysteine). Recently, we found hyperhomocysteinemia to be a risk factor for recurrent venous thrombosis in patients between 20 and 70 years of age, as compared with controls from the general population.[7] Although the results of these studies support the hypothesis that mild hyperhomocysteinemia is a risk factor for venous thrombosis, the studies were not designed to estimate the risk in the general population.

We measured homocysteine concentrations in patients and matched control subjects participating in the Leiden Thrombophilia Study.[8-11] This is a population-based case–control study designed to measure the effect of several acquired and genetic risk factors for thrombosis in the general population. Because of the data available on the study subjects, we were able to investigate whether the effect of hyperhomocysteinemia was independent of other well-established risk factors for thrombosis, such as a deficiency of protein C, protein S, or antithrombin; use of oral contraceptives; and pregnancy or recent childbirth. Recently, resistance to activated protein C caused by a single point mutation in the factor V gene (factor V Leiden) has been reported to be the most common hereditary cause of venous thrombosis.[12] Since hyperhomocysteinemia also appears to be common, we examined the risk of thrombosis in persons with both abnormalities.

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METHODS

The methods by which blood samples were obtained and interview data were collected have been described elsewhere.[8-11] The study protocol was approved by the local ethics committee, and all participants gave their informed consent. Briefly, consecutive patients younger than 70 years of age who had a first episode of deep-vein thrombosis, objectively confirmed (by impedance plethysmography, Doppler ultrasonography, compression ultrasonography, or contrast venography), between 1988 and 1993 and who had no known cancer were selected from the files of three anticoagulation clinics in the Netherlands (in Leiden, Amsterdam, and Rotterdam). These clinics monitor the anticoagulant treatment of virtually all patients in well-defined geographic areas. Each patient was asked to find his or her own healthy control subject of the same sex and age (within five years) by asking neighbors or friends. We restricted the present analysis to case patients and controls who were seen at the Leiden Anticoagulation Clinic and whose blood samples were processed and frozen on site with minimal delay. (Blood samples from participants in Amsterdam and Rotterdam were also processed in Leiden, which caused delays of several hours, and homocysteine measurements were therefore less accurate than those measured in samples from subjects in Leiden.[14])

The total homocysteine concentration was measured in citrated plasma by automated high-performance liquid chromatography with reverse-phase separation and fluorescent detection (with a Gilson 232-401 sample processor, Spectra-Physics 8800 solvent-delivery system, and Spectra-Physics LC 304 fluorometer). We used the method described by Fiskerstrand et al.[13] with some modifications.[11] If not otherwise stated, hyperhomocysteinemia was defined as a homocysteine level above the 95th percentile in the control group (18.5 μmol per liter).

We calculated matched odds ratios as estimates of the relative risk of thrombosis for homocysteine values above a given point, with the matching factor taken into account. The univariate matched odds ratio is the ratio of the number of pairs of case patients and controls in which the homocysteine value for the case patient was above the specified level and the value for the control was below that level to the number of pairs in which the homocysteine value for the control was above the specified level and the value for the case patient was below that level. The 95 percent confidence intervals were calculated from

a conditional logistic-regression algorithm by the maximum-likelihood method, with Egret software. We also investigated a possible dose–response relation by calculating odds ratios for several ranges of homocysteine concentrations in a conditional logistic model. In addition, we calculated odds ratios for men and women separately and for several age groups in order to study possible differences in risk among these subgroups.

We further explored the differences in risk between men and women by taking risk factors specific to women into account — specifically, the use of oral contraceptives, pregnancy, and recent childbirth. We analyzed the risk of thrombosis among women less than 50 years old, both with and without the inclusion of women with these risk factors, by calculating unmatched odds ratios. The use of unmatched odds ratios was necessary because in the restricted groups many matched pairs would not have been complete. Since the matched and unmatched odds ratios did not differ substantially in any of our analyses, we considered this approach justified.

We also assessed whether the increased risk associated with hyperhomocysteinemia in both sexes was confounded by other risk factors, such as a deficiency of protein C, protein S, or antithrombin. We repeated the analysis after excluding subjects with abnormally low levels of these proteins (measured, as previously reported, with a single test[8]) and estimated the risk associated with hyperhomocysteinemia in persons with normal protein C, protein S, and antithrombin levels.

Finally, we looked at the possibility of an interaction between hyperhomocysteinemia and heterozygosity (carrier status) for factor V Leiden, a rather common defect that causes resistance to activated protein C. We analyzed this interaction by calculating univariate odds ratios for thrombosis in persons with both or either of these risk factors, as compared with persons with neither risk factor.

Results

The ratio of male to female subjects among both the case patients and the controls was 1:1.3, and the mean age was 44 years (range, 16 to 70 for the case patients and 16 to 71 for the controls); both these variables were used in matching the case patients and the controls.

The median plasma homocysteine level in the patients was 12.9 μmol per liter (range, 4.8 to 60.2), and that in the controls was 12.3 μmol per liter (range, 6.4 to 37.5). The homocysteine concentrations of individual case patients and controls are shown in Figure 1.

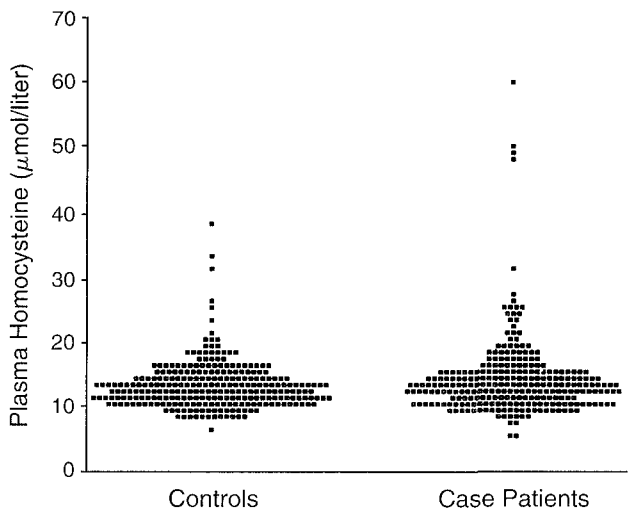

Figure 1. Plasma Homocysteine Levels in 269 Patients with Deep-Vein Thrombosis and 269 Controls.
Values shown have been rounded.

The 95th percentile of the homocysteine levels in the control group was 18.5 μmol per liter. Of the 269 patients, 28 (10 percent) exceeded this cutoff, as compared with 13 (5 percent, by definition) in the control group. The matched odds ratio for deep-vein thrombosis in subjects with a homocysteine concentration above the 95th percentile, as compared with those whose homocysteine levels were at or below that value, was 2.5 (95 percent confidence interval, 1.2 to 5.2). When the cutoff was set at the 90th percentile, the matched odds ratio was 1.9 (95 percent confidence interval, 1.1 to 3.3); it was 4.0 (95 percent confidence interval, 1.4 to 12.0) when the cutoff was the 97.5th percentile (Table 1).

In order to evaluate the possibility of a dose–response relation, we stratified the patients and controls according to their homocysteine concentrations and calculated odds ratios for thrombosis in the patients at the higher levels as compared with those at the lowest level. As Figure 2 shows, the risk of thrombosis did not increase among subjects with homocysteine levels up to 18 μmol per liter; the risk was greatly increased above 22 μmol per liter, however, indicating a threshold effect rather than a continuous dose–response relation.

Odds ratios for several age groups and for men and women separately are shown in Table 2. For both sexes, there was a sharp increase in the risk of thrombosis associated with hyperhomocysteinemia at increasing ages. The overall odds ratio for thrombosis associated with hyperhomocysteinemia in women was 7.0 (95 percent confidence interval, 1.6 to 30.8), and in men it was 1.4 (95 percent confidence interval, 0.6 to 3.4), with the cutoff set at the 95th percentile of the homocysteine levels in the control group ($P = 0.067$ for the comparison between the sexes). When we calculated the 95th percentile of the distribution of homocysteine levels for men and women separately, we found a 95th percentile of 17.1 μmol per liter among women and 20.0 μmol per liter among men in the control group. Using these cutoffs for hyperhomocysteinemia, we found an odds ratio for thrombosis of 3.8 (95 percent confidence interval, 1.4 to 10.2) for women and 1.8 (95 percent confidence interval, 0.6 to 5.4) for men.

The higher rate of hyperhomocysteinemia in women than in men was present at all ages, making it unlikely that the difference was due to risk factors specific to women, such as the use of oral contraceptives, recent childbirth, or pregnancy. Indeed, when we excluded women with these risk factors, the unmatched odds ratio for thrombosis that was associated with hyperhomocysteinemia (with the 95th percentile for both sexes — 18.5 μmol per liter — as the cutoff for hyperhomocysteinemia) among women under the age of 50 was 11.3 (95 percent confidence interval, 2.7 to 46.0), whereas it was 2.8 (95 percent confidence interval, 0.9 to 8.7) for all women, both those with and those without these risk factors, under the age of 50.

Of the 269 patients, 15 had protein C deficiency, 7 had protein S deficiency, and 10 had antithrombin deficiency. In the control group, four had protein C deficiency,

Table 1. Pairwise Distribution of Plasma Homocysteine Values in 269 Case Patients and Their Matched Controls, According to Various Definitions of Hyperhomocysteinemia.*

CUTOFF 90TH PERCENTILE (16.6 μmol per liter)		
CASE PATIENTS	CONTROLS	
	Above cutoff	Below cutoff
Above cutoff	6 pairs	38 pairs
Below cutoff	20 pairs	205 pairs

Matched odds ratio for thrombosis, 1.9 (95% CI, 1.1–3.3)

CUTOFF 95TH PERCENTILE (18.5 μmol per liter)		
CASE PATIENTS	CONTROLS	
	Above cutoff	Below cutoff
Above cutoff	3 pairs	25 pairs
Below cutoff	10 pairs	231 pairs

Matched odds ratio for thrombosis, 2.5 (95% CI, 1.2–5.2)

CUTOFF 97.5TH PERCENTILE (21.1 μmol per liter)		
CASE PATIENTS	CONTROLS	
	Above cutoff	Below cutoff
Above cutoff	2 pairs	16 pairs
Below cutoff	4 pairs	247 pairs

Matched odds ratio for thrombosis, 4.0 (95% CI, 1.4–12.0)

*For each cutoff point, subjects classified as having hyperhomocysteinemia were those with plasma homocysteine levels above the cutoff value, and subjects classified as not having hyperhomocysteinemia were those with levels at or below the cutoff value ("below cutoff"). The percentiles used as cutoffs were for the distribution of homocysteine values in the control group. Odds ratios were calculated as the risk of thrombosis in the subjects with hyperhomocysteinemia as compared with that in the subjects without hyperhomocysteinemia. CI denotes confidence interval.

eight had protein S deficiency, and eight had antithrombin deficiency. After excluding these subjects, we found a matched odds ratio for deep-vein thrombosis of 2.6 (95 percent confidence interval, 1.2 to 5.9), as compared with 2.5 (95 percent confidence interval, 1.2 to 5.2) when those subjects were included; this result shows that the effect of homocysteine is largely independent of these deficiencies in clotting-factor inhibitors.

With respect to the combination of factor V Leiden and hyperhomocysteinemia, we calculated odds ratios for thrombosis in subjects with both risk factors or either one in relation to subjects with neither. A total of 47 of the patients carried the factor V Leiden mutation, as compared with 7 of the controls. The small number with both defects made the results statistically unstable and somewhat sensitive to the cutoff chosen for elevated homocysteine levels. When the 90th percentile was used as the cutoff, the odds ratio for thrombosis associated with the presence of both risk factors (factor V Leiden and hyperhomocysteinemia) was 3.5 (95 percent confidence interval, 0.7 to 16.9); the odds ratios for thrombosis associated with factor V Leiden alone and hyperhomocysteinemia alone, calculated separately, were 9.5 and 2.2, respectively. With the 95th percentile used as the cutoff, the odds ratio for the combination of risk factors was 2.0 (95 percent confidence interval, 0.4 to 10.9), whereas the odds ratios for each risk factor separately remained virtually unchanged. The statistical uncertainty of results based on these data is reflected in the wide confidence intervals, which do not exclude a relative risk as high as 16.9.

DISCUSSION

Our study shows that hyperhomocysteinemia is a risk factor for deep-vein thrombosis in the general population. Moreover, our results suggest that the association between mild hyperhomocysteinemia and venous thrombosis is similar in degree to that reported for hyperhomocysteinemia and arterial vascular disease.[15,16] An unexpected finding was the substantial increase in the risk of thrombosis at the highest plasma homocysteine levels. Our data suggest that there may be a threshold level above which homocysteine has a thrombogenic effect.

Falcon et al. reported that hyperhomocysteinemia was a risk factor for juvenile thrombosis.[6] Our data imply that hyperhomocysteinemia is a risk factor for thrombosis in adult subjects as well, since we found an increasing odds ratio with increasing age.

When we analyzed men and women separately, we found a difference in the risk of thrombosis associated with hyperhomocysteinemia. Even when we used different cutoff points for hyperhomocysteinemia in men and women by calculating the 95th percentiles of their homocysteine distributions in the control group separately, we found that the odds ratio was roughly twice as high for women as for men. This suggests that women may be more susceptible to the pathologic effects of elevated homocysteine levels, even though their homocysteine levels are in general lower than those of men.[1] This effect cannot be explained by risk factors specific to women (such as pregnancy, recent childbirth, and oral-contraceptive use); an effect of these risk factors was unlikely in any case because the difference between men and women who did not have such risk factors was even more pronounced.

Hyperhomocysteinemia remained a risk factor for

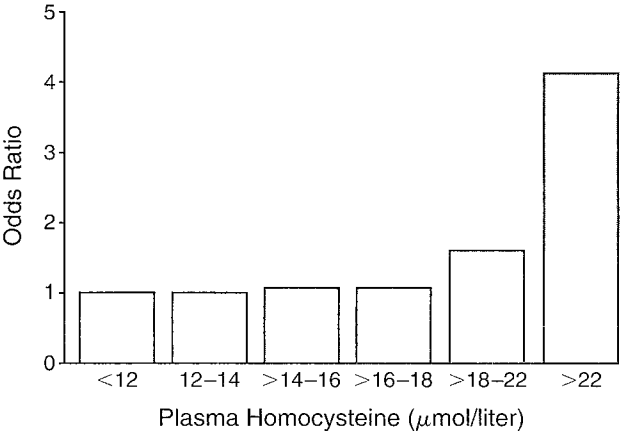

Figure 2 Odds Ratio for Thrombosis According to Plasma Homocysteine Level.

The reference category was the subjects with plasma homocysteine values of <12 μmol per liter.

Table 2. Odds Ratios for Thrombosis Associated with Hyperhomocysteinemia, According to Age and Sex.[a]

Age (yr)	Men	Women	Both Sexes
	odds ratio (95% CI)		
<30	0.5 (0.1–5.5)	1.0 (0.1–16.0)	0.7 (0.1–4.0)
30–50	1.3 (0.3–4.6)	7.0 (0.9–56.9)	2.4 (0.8–6.8)
≥50	2.5 (0.5–12.9)	∞[b]	5.5 (1.2–24.8)
All ages	1.4 (0.6–3.4)	7.0 (1.6–30.8)	2.5 (1.2–5.2)

[a] Odds ratios were calculated as the risk of deep vein thrombosis in subjects with hyperhomocysteinemia (defined as a homocysteine level above the 95th percentile in the control group [18.5 μmol per liter]) as compared with the risk in those without hyperhomocysteinemia. CI denotes confidence interval.

[b] The odds ratio was 12.0 (95 percent confidence interval 1.6 to 92.3) when the cutoff used was the 90th percentile in the control group.

deep-vein thrombosis after we excluded subjects with other well-established risk factors; that is, the association with thrombosis was not explained by the presence of other hereditary risk factors for thrombosis, such as a deficiency of protein C, protein S, or antithrombin. The same was true of the most common hereditary risk factor for deep venous thrombosis, resistance to activated protein C, since hyperhomocysteinemia also increased the risk of thrombosis in those without this abnormality. We investigated a possible interaction between resistance to activated protein C (factor V Leiden) and hyperhomocysteinemia. Although we found that the risk of thrombosis may be higher in carriers of the mutation who have hyperhomocysteinemia than in noncarriers with hyperhomocysteinemia, the combined effect in our subjects seemed smaller than for factor V Leiden alone. Because of the small numbers involved, the only reasonable conclusion is that the two factors do not potentiate each other.

Many hypotheses have been proposed to explain how hyperhomocysteinemia may lead to venous thrombosis and atherosclerosis. One hypothesis is that homocysteine has a toxic effect on the vascular endothelium and on the clotting cascade.[1,2] Several in vitro studies seem to support this view.[17,18] However, virtually all these studies used amounts of homocysteine that produced higher-than-physiologic concentrations. Alternatively, hyperhomocysteinemia may reflect abnormal methionine metabolism that affects the methylation of DNA and cell membranes.[19]

Elevated homocysteine levels may result from low levels of folic acid, vitamin B_6, or vitamin B_{12}. Moreover, several genetic alterations in enzymes involved in homocysteine metabolism have been described.[20-22] It remains unclear whether hyperhomocysteinemia of different causes entails the same risk of thrombosis. Nevertheless, it is well known that vitamin supplementation lowers homocysteine concentrations in almost all subjects with hyperhomocysteinemia, regardless of the underlying cause.

We conclude that mild hyperhomocysteinemia is a risk factor for deep-vein thrombosis in the general population. The next question to be answered is whether homocysteine-lowering therapy — folic acid, vitamin B_6, or vitamin B_{12} — contributes to the prevention of recurrent venous thrombosis.[23-25]

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