Plasma Homocyst(e)ine and Arterial Occlusive Diseases: A Mini-Review

M. René Malinow

Homocysteine (HCY), which is derived from the intracellular metabolism of methionine, is exported into plasma, where it circulates mostly in oxidized forms (i.e., homocysteine and cysteine-HCY disulfide) and mainly bound to proteins. Concentrations of total HCY, or homocyst(e)ine [H(e)], are increased in 15–40% of patients with coronary, cerebral, or peripheral arterial diseases. Such association of H(e) with arterial occlusive diseases has been documented in retrospective, cross-sectional, and prospective studies. Concentrations of H(e) are also increased in subjects having thickened carotid arteries, as determined by ultrasonography, and who are asymptomatic for atherosclerosis. Statistical analyses of data from several series of patients demonstrate that H(e) concentrations are associated with coronary artery disease, independently from most other risk factors for atherosclerosis. The increased concentrations of H(e) are readily corrected by folic acid, occasionally supplemented with pyridoxine, vitamin B₁₂, choline, or betaine. Whether these supplements affect the evolution of atherosclerotic disease needs to be established by prospective, placebo-controlled clinical trials.

Indexing Terms: coronary artery disease/stroke/carotid wall thickening/intermittent claudication/amino acids

Homocystinuria, a rare genetic abnormality, shows severe increases of homocyst(e)ine [H(e)] concentrations in blood.¹ This disorder is associated with thromboembolic complications at an early age, which may result in stroke, myocardial infarction, renovascular hypertension, intermittent claudication, mesenteric ischemia, and pulmonary embolism. Such patients may also exhibit mental retardation and other abnormalities resembling the unrelated Marfan syndrome (i.e., ectopia lentis and skeletal deformities) (1).

Plasma total homocysteine, by convention called "homocyst(e)ine," is the sum of the thiol-containing amino acid, homocysteine, and the homocysteiny1 moiety of the disulfides homocysteine and cysteine–homocysteine, whether free or bound to protein (see Fig. 1). The concentration of H(e) in blood, plasma, or serum, i.e., homocyst(e)ine, is ordinarily <16 μmol/L in plasma. In hyperhomocyst(e)inemia (2) the concentration of H(e) is above "normal": moderate, intermediate, and severe hyperhomocyst(e)inemas refer to plasma concentrations between 16 and 30, 31 and 100, and >100 μmol/L, respectively (3).

Hyperhomocyst(e)inemia may be associated with defective enzymes involved in the metabolism of methionine, mostly cystathionine β-synthase (1) or methylenetetrahydrofolate reductase (4). Moreover, there is a negative correlation between the concentration of H(e) and the intake and blood concentrations of folic acid, vitamin B₁₂, and vitamin B₆ (5–8). High concentrations of homocysteine change hemostatic conditions from antithrombotic to thrombogenic (9–16), induce oxidation of low-density lipoprotein (17), and increase incorporation of lipoprotein(a) into fibrin (18). These mechanisms may be related to the pathogenesis of atherothrombosis in hyperhomocyst(e)inemia.

Here I review certain epidemiological observations on the association between plasma H(e) concentrations and arterial occlusive diseases in adults who lack the mental and skeletal characteristics of homocystinuria. Numerous investigators have reported on H(e) concentrations in atherosclerotic patients (6, 19). I will refer mostly to studies in which the basal values for H(e) were measured at the Oregon Regional Primate Research Center by HPLC with electrochemical detection (2, 20). This procedure compares favorably with other methods (6), e.g., assays involving derivatized S-adenosylmethionine (21–23), gas chromatography–mass spectrometry (24), and derivatization with fluorogenic reagents for thiols followed by HPLC with fluorescence detection (25–29).

Several studies suggest that the prevalence of abnormal concentrations of basal plasma H(e) is not much different from those seen after post-methionine-loading tests (19).

Homocyst(e)inemia in Coronary Artery Disease (CAD)

Wilcken and Wilcken (30) first described increased concentrations of cysteine–homocysteine disulfide after methionine loading in CAD patients. Murphy-Clutton et al. (31), and Clarke et al. (32) also reported increased homocysteine species post-methionine-loading in similar patients. Kang et al. (33), Israelsson et al. (34), and Ubbink et al. (35) found increased basal concentrations of H(e) in CAD subjects.

Several colleagues and I measured basal H(e) in 405 consecutive patients attending an internist's office (20). The age of men and women with CAD was 64.5 ± 9.6 (SD) and 70.1 ± 8.2 years, respectively, compared with 56.6 ± 11.3 and 62.4 ± 11.5 years in the controls. The patients with CAD had higher H(e) concentrations than the control subjects, i.e., 13.07 ± 4.32 vs 11.21 ± 3.71 μmol/L in men (P = 0.02), and 12.97 ± 7.39 vs 10.15 ± 4.99 μmol/L in women (P = 0.03; In-transformed values and age as covariate). About 20% of male coronary pa-
tients had H(e) concentrations exceeding the 95th percentile distribution of the control subjects. In another study (36), we established the presence of CAD by coronary angiography in 175 men (ages 50 ± 7 years) and contrasted their H(e) concentrations with those in 255 control subjects (ages 49 ± 6 years) clinically free of CAD. Concentrations were greater in the former: 13.66 ± 6.44 vs 10.95 ± 4.92 μmol/L (P <0.001). Stepwise discriminant analysis on cases vs controls was performed with models incorporating risk factors for atherosclerosis and using a forward/backward procedure. Smoking, high-density lipoprotein cholesterol, hypertension, H(e), and diabetes were associated (P <0.05) in descending order with the presence of CAD in the first model. In a second model, apolipoproteins B and A-I were used and H(e) remained associated with the presence of CAD. These data suggested that H(e) was an independent risk factor for atherosclerosis (36). Families of 71 of the CAD patients were selected on the basis of availability of relatives. In 20 families (28%), the proband had H(e) concentrations greater than the 90th percentile of controls; familial segregation was observed in 10 of these kindred, which suggests the likelihood of familial hyperhomocyst(e)inemia (37).

To test familial correlation, we also measured H(e) in plasma samples from 37 men and women with early familial coronary heart disease and in 48 controls matched for age and sex (38). The subjects were selected to include 13 male sibling pairs with CAD as well as 13 male sibling pairs and 13 spouse pairs as controls (all matched for age). The mean H(e) concentrations were significantly higher in CAD patients, both in men (14.31 vs 11.09 μmol/L; P = 0.02) and women (9.51 vs 7.40 μmol/L; P = 0.02). A strong familial correlation of plasma H(e) was observed among all 26 male sibling pairs (r = 0.52; P <0.01) and was present separately in subjects with CAD as well as in control siblings. The data suggested that hyperhomocyst(e)inemia was an inherited abnormality that might explain certain cases of early familial CAD. A further case-control study was performed on 199 men with CAD (ages 50.3 ± 5.2 years) and in 156agematched control subjects (49.0 ± 7.4 years) (39). The extent of CAD was established by coronary angiography. The geometric mean concentrations of H(e) were significantly higher in patients than in controls: 8.9 vs 7.8 μmol/L, respectively (P <0.001). This difference remained significant on multiple logistic analysis after adjusting for body mass index, systolic blood pressure, serum cholesterol, and high-density lipoprotein cholesterol, but not after additional adjustment for fibrinogen. There was a positive correlation between H(e) and fibrinogen concentrations; if this association was causal, perhaps high concentrations of H(e) lead to the release of fibrinogen into plasma. Given that homocysteine inhibits thrombomodulin and Protein C activation (11), perhaps leading to increased production of thrombin, these mechanisms, which are associated with increased production of fibrinogen, may favor thrombosis (39).

Findings in the previous retrospective studies do not allow us to decide whether high concentrations of H(e) are a consequence of the arterial occlusive process or not. Prospective studies should be able to shed light in this respect. We measured H(e) concentrations as part of the Physician's Health Study, an ongoing, randomized, double-blind, placebo-controlled 2 × 2 factorial trial of acetylsalicylate and β-carotene (40). A total of 22 071 US male physicians, ages 40 to 80 years in 1982, were enrolled. Blood samples were obtained in 14 916 subjects at entry; in the next 5 years, 271 cases of nonfatal myocardial infarction or fatal outcomes were ascertained by autopsy or medical records. These cases were matched to 271 controls free from myocardial infarction at the time of the case's diagnosis. H(e) concentrations in the 271 cases exceeded those in the controls (11.1 ± 4.0 vs 10.5 ± 2.8 μmol/L, respectively; P <0.03). Contrasting the top 5% with the lower 90% of the control distribution in a matched conditional logistic regression model and adjusting for age and smoking, we determined that the relative risk for myocardial infarction was 3.1 (95% confidence interval [CI] 1.4–6.9). After further adjustment for several risk factors and assignment of acetylsalicylate, the relative risk was 3.4 (95% CI, 1.3–8.8) (41).

Homocyst(e)inemia in Cerebrovascular Disease

High concentrations of H(e) have also been observed in patients with cerebrovascular disease (32, 42–44). We previously reported that 41 patients with acute strokes and 27 patients with transient ischemic attacks had higher mean concentrations of H(e) than did 31 controls. About one-third of the patients had H(e) concentrations higher than the controls did. We found no relation between H(e) concentration and other recognized stroke risk factors or stroke type; however, concentrations of serum uric acid and plasma H(e) were positively correlated (45).

In 142 survivors of stroke, mean H(e) concentrations were greater than in 66 controls, and hyperhomocyst(e)inemia was present in 40% of the stroke patients and in 6% of the controls. The H(e) concentrations were increased in patients with lacunar, hemorrhagic, or embolic strokes. Plasma H(e) showed no significant association with the presence of hypertension, smoking, or
hypercholesterolemia or with the concentration of blood glucose, glycohemoglobin, or plasma fibrinogen. About 40% of the H(e) variance could be predicted by the values of blood folate, plasma pyridoxal 5-phosphate, and serum creatinine. Thus, hyperhomocyst(e)inemia seemed to be partly related to renal function and to the concentration of cofactors involved in the metabolism of homocysteine (46).

Homocyst(e)inemia in Peripheral Arterial Disease

Boers et al. (42) and Clarke et al. (32) found high concentrations of plasma H(e) in subjects with peripheral arterial occlusive disease. We studied 47 patients with this disease and found carotid arterial involvement in 35 and iliofemoral lesions in 32 subjects; most patients had both arterial territories involved. Plasma H(e) in these patients was 16.16 ± 6.94 µmol/L vs 10.10 ± 2.16 µmol/L in controls (P <0.05) (2).

We also studied 78 patients with intermittent claudication, selected from an epidemiological survey of all middle-aged men (n = 15,253, ages 45–69 years) in Linköping County, Sweden; as controls, 98 age-matched but otherwise randomly selected men were included. Concentration of H(e) was significantly higher in the affected subjects than in controls (16.74 ± 5.45 vs 13.80 ± 3.21 µmol/L; P <0.0002); 23% of the patients had H(e) concentrations above the 95th percentile of the controls. The H(e) concentrations were independent of other risk factors for peripheral atherosclerotic disease, but increased H(e) was observed mainly in subjects with low concentrations of serum folate (47).

Homocyst(e)inemia in Subclinical Atherosclerosis

We reported the correlation of H(e) to carotid arterial wall intimal-medial thickness (measured by high resolution B-mode ultrasound imaging) in 287 case-control pairs of individuals free of clinical atherosclerosis (48). These subjects were being followed in the Atherosclerosis Risk in Communities (ARIC) Study, which has a cohort component in which cardiovascular disease end points and risk factors are being assessed in 15,800 men and women between the ages of 45 and 64, selected as a probability sample from four locations: Forsyth County, NC; Jackson, MS; Minneapolis suburbs, MN; and Washington County, MD (49). For our analysis we used cross-sectional data from the baseline visit, which took place between fall 1986 and winter 1989.

Among subjects without history of arterial occlusive disease (e.g., angina, heart attack, transient ischemic attack, stroke, or intermittent claudication) who satisfied the minimum visualization requirements for the carotid arteries, the cases and controls were defined on the basis of intimal-medial thickness of the carotid arteries measured by B-mode ultrasound. Affected subjects had at least two unilateral measurements of the common carotid artery far-wall thickness >2.5 mm or bilateral thickening corresponding to a maximum intimal-medial thickness of ≥1.7 mm in the internal carotid and (or) ≥1.8 mm in the carotid bifurcation and (or) ≥1.6 mm in the common carotid arteries. These cutoff points exceeded the 90th percentiles for the respective values in the ARIC cohort. As expected, average intimal-medial carotid artery wall thickness for the six carotid sites was greater for the affected cases (1.21 mm) than for controls (0.63 mm) (P <0.0001); the mean homocyst(e)ine concentrations were 9.26 and 3.82 µmol/L, respectively (P <0.001).

The odds ratios for having a thickened carotid arterial wall according to H(e) concentrations were adjusted by design and analysis for all matching criteria (sex, race, age group, clinic center, and visit date). For the total 287 pairs, an increase of 1 SD in H(e) (3.2 µmol/L) was associated with a 35% increase in the odds of being affected. The odds ratio for the highest quintile of H(e) compared with the lowest quintile was 3.15 (95% CI, 1.57–6.33) (48).

In conclusion, high concentrations of H(e) are frequently observed in patients with coronary, cerebrovascular, or peripheral arterial diseases, as well as in subjects with subclinical atherosclerosis. Although homocyst(e)inemia may be decreased rapidly by the administration of folate—occasionally accompanied by vitamin B12, vitamin B6, choline, or betaine (50)—it is important to determine whether the clinical evolution of arterial occlusive diseases is influenced by those supplements. Such data must await the outcome of prospective, placebo-controlled clinical trials.

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References


17. Pathasarathy S. Oxidation of low-density lipoprotein by thiol compounds leads to its recognition by the acetyl LDL receptor. Biochim Biophys Acta 1987;917:337–40.


37. Genest JJ, McMamara MT, Upson B, Salem DN, Ordovas JM, Schaefer EJ, Malinow MR. Prevalence of familial hyperhomo-


46. Brattstrom L, Lindgren A, Iversen B, Malinow MR, Norrv-

