Carotid Artery Intimal-Medial Wall Thickening and Plasma Homocyst(e)ine in Asymptomatic Adults

The Atherosclerosis Risk in Communities Study

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Background. Plasma levels of homocyst(e)ine are elevated in certain patients with occlusive arterial diseases. We extended these findings to asymptomatic adults.

Methods and Results. We determined plasma homocyst(e)ine levels in 287 pairs of asymptomatic adults. Cases and controls were defined on the basis of intimal-medial thickness of the carotid wall as measured by B-mode ultrasound. Study subjects had no history of atherosclerotic disease and were selected from a probability sample of 15,800 men and women between 45 and 64 years old. Subjects with thickened intimal-medial carotid walls (cases) had higher plasma homocyst(e)ine levels than controls ($p<0.001$). The odds ratio for having a thickened carotid artery wall was 3.15 ($p<0.001$) for subjects in the top quintile of plasma homocyst(e)ine levels ($>10.5 \mu\text{mol/L}$) compared with those in the bottom quintile ($<5.88 \mu\text{mol/L}$).

Conclusions. The present study as well as observations on the common occurrence of elevated plasma homocyst(e)ine levels in patients with occlusive arterial diseases suggest that clinical trials should be conducted to determine whether normalization of hyperhomocyst(e)inemia may prevent progression of atherosclerosis. (Circulation 1993;87:1107-1113)

Key Words • homocyst(e)ine • atherosclerosis • epidemiology • case-control studies • cardiovascular disease

Several have reported the occurrence of elevated plasma levels of total homocyst(e)ine or of disulfides derived from homocysteine in a large proportion of patients with cerebrovascular or peripheral arterial diseases.1-6 However, these observations on symptomatic patients do not exclude the possibility that symptomatic arterial occlusive disease or the changes in lifestyle or medications associated with it may influence the levels of these sulfur-containing amino acids. We report a study of the relation of plasma homocyst(e)ine levels to carotid arterial wall intimal-medial thickness measured by high resolution B-mode ultrasound imaging in asymptomatic individuals. There is reason to believe that carotid arterial wall thickening reflects atherosclerosis.7-9

Methods

The design and objectives of the Atherosclerosis Risk in Communities (ARIC) Study have been described.10 Only the main features will be mentioned here. The ARIC Study has a cohort component in which cardiovascular disease end points and risk factors are assessed on 15,800 men and women between the ages of 45 and 64 years selected as a probability sample from four locations: Forsyth County, N.C.; Jackson, Miss.; Minneapolis suburbs, Minn.; and Washington County, Md. The present analysis uses cross-sectional data from the baseline visit, which took place between Fall 1986 and Winter 1989.

The ARIC Study participants include urban, suburban, and rural residents, both men and women and blacks and whites. Details on sampling, recruitment, and clinic examination as well as blood analyses have been reported.10 Hypertension was defined as resting blood pressure taken by random zero sphygmomanometry of $>140 \text{ mm Hg}$ systolic and/or $90 \text{ mm Hg}$ diastolic or the current use of antihypertensive medication.

Ultrasound Imaging

The ultrasound methods for carotid artery imaging are based on the technique described by Pignoli et al9
(Figures 1 and 2). The scanning protocol is common to the four centers. Technicians are trained and certified at the ARIC Ultrasound Reading Center. Ultrasound studies are read in a central laboratory according to a standardized protocol. The present observation includes measurement of the B-mode ultrasound image of intimal-medial thickness of the carotid arteries at six different sites (common, bifurcation, and internal in each side) and one side of the popliteal artery, as described previously. Interobserver and intraobserver variability of measurements has been reported.

Among subjects without history of arterial occlusive disease (e.g., angina, heart attack, transient ischemic attack, stroke, or intermittent claudication) who satisfy minimum visualization requirements for the carotid arteries, cases and controls were defined on the basis of the thickness of intimal-medial thickness of the carotid arteries as measured by B-mode ultrasound. Cases had at least two unilateral measurements of the common carotid artery far-wall thickness \( \geq 2.5 \) mm or bilateral thickening corresponding to a maximum intimal-medial thickness of \( \geq 1.7 \) mm in the internal carotid and/or \( \geq 1.8 \) mm in the carotid bifurcation and/or \( \geq 1.6 \) mm in the common carotid arteries. These cutoff points exceed the 90th percentiles for the respective values in the ARIC cohort.

Paired controls were selected among subjects without evidence of carotid thickening, i.e., a maximum near-wall thickness \( < 1.2 \) mm at any site and a maximum far-wall thickness \( < 1.0 \) mm at the internal and common carotid and popliteal and \( < 1.2 \) mm at the carotid bifurcation. These values correspond approximately to the 75th percentile of intimal-medial on all carotid and popliteal segments visualized. Controls were paired to cases within strata defined by study center, race, sex, 10-year age groups, and 6-month examination period. Controls first were searched among individuals with the largest number of carotid segments visualized and then from this group, individuals were sought with close date of examination. If more than one control were available in any stratum, one was chosen at random.

Sample calculations suggested that a sample size of 250–300 case–control pairs would provide adequate power to achieve the objectives in this study. Thus, 310

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**Figure 1.** B-mode ultrasonogram of right carotid bifurcation: control. Boundary 4, far-wall lumen intima interface. Boundary 5, far-wall media-adventitia interface. Intima plus media, 1.1 mm. Cursor (0.9 mm) is at level of flow divider. Biosound 2000 II s.a. (8 MHz).

**Figure 2.** B-mode ultrasonogram of right carotid bifurcation: case. Boundary 4, far-wall lumen intima interface. Boundary 5, far-wall media-adventitia interface. Intima plus media, 3.1 mm. Cursor (0.9 mm) is at level of flow divider. Biosound 2000 II s.a. (8 MHz).
pairs were randomly selected among the 492 case-control pairs originally identified. Serum samples were available for homocyst(e)ine determination in 287 of these 310 pairs. There were no statistically significant differences between the pairs with or without homocyst(e)ine determination according to any of the main variables used in this study.

Homocyst(e)ine Determination

Venous blood samples were anticoagulated with sodium citrate and centrifuged, and plasma was stored at –70°C until analysis. In 1991, the paired samples were coded and sent on dry ice to the laboratory in Oregon for “blind” determination of homocyst(e)ine. Homocyst(e)ine, i.e., the sum of homocysteine and the homocysteiny l moieties of the disulfides, homocystine and cysteine-homocysteine, whether in free or bound forms, was assayed by high-pressure liquid chromatography and electrochemical detection, based on the method of Smolin and Schneider, as previously described with minor modifications. Results were expressed as homocysteine in micromoles per liter of the mean of two duplicates; one of the duplicates contained an internal standard of N-acetyl-DL homocysteine thiolactone (Fluka Chemical Corp., Ronkonkoma, N.Y.) to detect possible errors in the handling of samples. Analyses were performed in paired case and control samples. Sixteen paired external quality control samples ("phantoms") from the ARIC Study were incorporated "blindly" into runs and were treated identically to participants' samples. The reliability coefficient (1 minus the proportion of the total variance attributable to laboratory variance) of the 16 "phantom" replicates was 0.93. This reliability coefficient can be interpreted as the correlation between replicate measurements. The coefficient of variation (standard deviation of the mean of the paired "phantom" replicates multiplied by 100 and divided by the mean) was 10.9%. In addition, three quality control samples were interspersed with the study samples each day, according to a strictly randomized procedure; these standards were from two large, well-mixed pools obtained from American Red Cross plasma. The mean value of homocyst(e)ine in the quality control samples was 16.50±2.03 (±SD) μmol/L (n=70). Homocyst(e)ine levels were adjusted to this mean in each daily run to account for day-to-day method variability; statistical analyses of the adjusted levels gave similar results as those using the unadjusted levels, so the adjusted values were not considered further.

Statistical Analysis

Mean values and proportions of all study variables were compared between cases and controls; however, because of matching, these mean values and proportions should be interpreted as data adjusted for the matching factors. Statistical significance of the differences in mean values between cases and controls was evaluated by paired t tests; differences in proportions were tested using McNemar's paired χ2 with Edward's correction for continuity. The Spearman rank correlation was used to assess the association between homocyst(e)ine values and other variables. Odds ratios for being a case were determined using conditional logistic regression based on matched pairs. All statistical analyses were carried out using SAS, version 6.04; statistical tests were two-tailed.

Results

The 287 case-control pairs of subjects were distributed in the four centers as follows: 27% from Washington County, Md.; 32% from Minneapolis suburbs, Minn.; 17% from Jackson, Miss.; and 24% from Forsyth County, N.C. Fifty-seven percent of the pairs were men, 82% were white, and 67% were ≥55 years old.

As shown in previous ARIC Study reports, cases had a higher prevalence of smoking and hypertension, higher levels of total and low density lipoprotein (LDL) cholesterol, triglycerides, and fibrinogen, and lower levels of high density lipoprotein (HDL) cholesterol. As expected, average intimal-medial carotid artery wall thickness for the six carotid sites was greater for cases (1.21 mm) than for controls (0.63 mm) (p<0.001); mean homocyst(e)ine level was 9.26 μmol/L among cases and 8.32 μmol/L among controls (p<0.001) (data not shown in the tables). The sample frequency distributions of homocyst(e)ine in controls and cases are shown in Figure 3; compared with controls, there was a displacement of cases to higher values. However, the sample size is too small to determine a bimodal distribution for cases.

The odds ratios for having a thickened carotid arterial wall according to homocyst(e)ine levels are presented in Table 1. These odds ratios are adjusted by design and analysis for all matching criteria (sex, race, age group, center, and visit date). For the total 287 pairs, an increase in 1 SD of homocyst(e)ine (3.2 μmol/L) was associated with a 35% increase in the odds of being a case. The increase in the odds of being a case associated with 1 SD increase in homocyst(e)ine was 62% in women and 51% in whites. The odds ratio for the highest quintile of homocyst(e)ine compared with the lowest quintile was 5.15 [95% confidence intervals (CI), 1.57–6.33]. All strata-specific results presented in Table 1 consistently showed an increase in the odds ratio with increasing homocyst(e)ine levels. The associations were stronger among women, whites, subjects with hypertension, and nonsmokers. The associations in blacks were weaker, but in the same direction as in whites; the sample size was small (only 52 pairs of black subjects). Tests for interactions among strata for all categorical
variables in Table 1 were not statistically significant (not shown in the table).

In our sample, homocyst(e)ine was found to show positive association with age, systolic and diastolic pressures, waist-to-hip ratio, and fibrinogen; HDL cholesterol showed inverse association. However, the complex sampling scheme used in this study makes inferences to the total population dubious; therefore, these results are not presented. Nevertheless, these analyses allowed us to identify potential confounders of the association between homocyst(e)ine and case−control status. Table 2 shows odds ratios with further adjustment for age (as a continuous variable), waist-to-hip ratio, HDL cholesterol, smoking, and systolic blood pressure. The associations in Table 2 are weaker than those in Table 1, suggesting that the association of homocyst(e)ine with carotid thickening was explained partially by other risk factors. However, an association was still present in all subgroups although no longer statistically significant. Further adjustment for fibrinogen levels did not change these results substantially.

**Discussion**

The present study showed that asymptomatic adult subjects with carotid artery wall thickening revealed by ultrasound images had higher levels of plasma homocyst(e)ine than controls. Although values of homocyst(e)ine in controls were somewhat lower than in

### Table 1. Paired Odds Ratios for Case Status According to Homocyst(e)ine by Sex, Race, Hypertension, and Current Smoking Status

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>OR (95% CI) for increase in 1-SD unit of H(e)*</th>
<th>OR¶ for quintiles of H(e) vs. 1st quintile**</th>
<th>LR test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>All subjects</td>
<td>1.35† (1.12, 1.62)</td>
<td>1.33</td>
<td>1.83</td>
</tr>
<tr>
<td>Men</td>
<td>1.22 (0.98, 1.51)</td>
<td>0.91</td>
<td>1.56</td>
</tr>
<tr>
<td>Women</td>
<td>1.62† (1.17, 2.58)</td>
<td>1.81</td>
<td>1.90</td>
</tr>
<tr>
<td>Whites</td>
<td>1.51‡ (1.20, 1.90)</td>
<td>1.30</td>
<td>2.38§</td>
</tr>
<tr>
<td>Blacks</td>
<td>1.11 (0.85, 1.45)</td>
<td>1.46</td>
<td>0.62</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.64† (1.15, 2.33)</td>
<td>0.88</td>
<td>2.01</td>
</tr>
<tr>
<td>No hypertension</td>
<td>1.17 (0.93, 1.46)</td>
<td>1.60</td>
<td>2.54§</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.18 (0.89, 1.57)</td>
<td>0.83</td>
<td>1.49</td>
</tr>
<tr>
<td>Not current smoker</td>
<td>1.23 (0.98, 1.53)</td>
<td>1.32</td>
<td>1.66</td>
</tr>
</tbody>
</table>

CI, confidence intervals; H(e), homocyst(e)ine; OR, odds ratios; LR, likelihood ratio.
*Sample SD of H(e) among controls: 3.20 μmol/L.
†p<0.01, ‡p<0.001, §p<0.05.
‡Blood pressure >140 mm Hg systolic and/or >90 mm Hg diastolic, or current antihypertensive treatment.
¶Quintiles based on the homocyst(e)ine distribution among controls. Cut-offs for quintiles: 5.88, 7.05, 8.26, 10.15 μmol/L, respectively.
**Likelihood ratio test (4 df) for the model with all four dummy variables for each quintile of homocyst(e)ine compared with the model with only the intercept.

Conditional logistic regression is based on 287 pairs.

### Table 2. Adjusted Odds Ratios for Case Status According to Quintiles of Homocyst(e)ine by Sex and Race

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>OR* for quintiles of H(e) vs. 1st quintile†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>All subjects</td>
<td>0.90</td>
</tr>
<tr>
<td>Men</td>
<td>0.46</td>
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<tr>
<td>Women</td>
<td>1.60</td>
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<tr>
<td>Whites</td>
<td>0.79</td>
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<tr>
<td>Blacks</td>
<td>1.92</td>
</tr>
</tbody>
</table>

OR, odds ratio; H(e), homocyst(e)ine.
*Simultaneously adjusted for age (continuous), waist-to-hip ratio, HDL cholesterol, smoking, and systolic blood pressure, in addition to quintile of sex, race, age group, center, and visit date.
†Quintiles based on the homocyst(e)ine distribution among controls. Cut-offs for quintiles: 5.88, 7.05, 8.26, 10.15 μmol/L, respectively.
‡p<0.05.

Conditional logistic regression based on 287 pairs.
controls from other series, proportionate changes probably also occurred in cases because pairs were analyzed concurrently. The ultrasound images may be interpreted on the basis of the observations of Pignoli et al. These authors established by in vitro and in vivo studies that a "double-line pattern" of arterial images could be observed in most ultrasound interrogations of carotid and aortic segments. The inner ultrasound reflection probably corresponded to the intima-blood interface because it disappeared when a small portion of the aortic wall was excised from the luminal side. The outer ultrasound reflection probably corresponded to the media-adventitia interface because the image disappeared when the adventitia and periartetial tissues were removed. There was good agreement between in vitro ultrasound and histological measurements of intimal-medial thickness. We estimated from their figures that increased intimal-medial thickness, measured histologically, better corresponded to changes of the intima than to the media. Thus, our ultrasound images probably reflect a thicker intima in cases than in controls. Furthermore, extrapolating from the histological findings of Pignoli et al in aortic segments, the thicker carotid images that defined cases are likely to be associated with the presence of internal fibromuscular cap with a lipid and/or necrotic core. There are suggestive indications that progression of asymptomatic carotid artery wall thickening to symptomatic arterial occlusive disease may take place. Accordingly, it has been shown that carotid intimal-medial wall thickness increases with age as well as with the presence of other traditional risk factors for atherosclerosis, such as serum lipids, cigarette smoking, and blood pressure levels. Furthermore, hypercholesterolemic patients who had thicker carotid intimal-medial wall than normocholesterolemic controls.

We have found that levels of homocyst(e)ine were positively correlated with age, waist-to-hip ratio, blood pressure, fibrinogen levels, and carotid wall thickness; there also were negative correlations between homocyst(e)ine and HDL cholesterol levels. In all instances, however, the correlations were weak. Logistic regression analysis demonstrated that the odds ratio for having carotid artery thickening increased monotonically with increasing homocyst(e)ine levels (Table 1). Odds ratios above unity, although at lower magnitudes, still were obtained after adjusting for age, HDL cholesterol, smoking, systolic blood pressure, and waist-to-hip ratio (Table 2) as well as levels of fibrinogen. These data suggest that homocyst(e)ine is a risk factor for carotid arterial wall thickening among subjects free of clinical atherosclerotic disease and that this association persists, albeit slightly weakened, after adjusting for established risk factors for atherosclerosis. This may be due to confounding or may stem from some of these risk factors being implicated or sharing the same pathophysiological mechanisms in the development of atherosclerosis as homocyst(e)ine. Because sample selection was made in terms of cases and controls, the actual risk of disease (caseness) cannot be estimated directly from the case-control data. However, it is clear that one can directly estimate the ratio of odds of high homocyst(e)ine level in cases compared with noncases, and it is well known that this exposure odds ratio actually is equal to the disease odds ratio, i.e., to the ratio of odds of disease among those with a high homocyst(e)ine level compared with the low level. It has been shown that the logistic regression methods that we used produce valid estimates of that odds ratio but not of absolute odds of disease. It is also known that when the disease is rare, the odds ratio is approximately equal to the risk ratio, so we are justified in using the term "risk factor" even though absolute risk cannot be estimated directly from the data. An independent association between homocyst(e)ine and other risk factors for atherosclerosis has been reported in retrospective and prospective studies of subjects with coronary artery disease.

The pathogenesis of hyperhomocyst(e)ineemia may involve genetic, nutritional, pharmacological, and other factors. The former include impaired activity of cystathionine β-synthase (EC 4.2.1.22) or of thermolabile methylene tetrahydrofolate reductase (EC 1.1.1.68). Certain hyperhomocyst(e)inemic atherosclerotic patients have been considered to be heterozygotes for abnormalities of those enzymes. Heritability estimates in 96 identical and 92 fraternal adult male twins suggested that strong genetic influences affect homocyst(e)ine levels and that environmental factors also may be involved. Moreover, familial segregation was observed in one half of probands with premature coronary artery disease also having homocyst(e)ine levels above the 90th percentile of the control group distribution. Nutritional factors that may be involved in the control of homocyst(e)ine levels include defects in the bioavailability of folate, pyridoxine, or vitamin B12. Elevated homocyst(e)ine levels also may result from pharmacological agents, such as celestipol/niacin therapy or methotrexate and nitrous oxide or from certain pathological conditions, such as renal failure.

It has been speculated that an increase in carotid wall thickness may reduce oxygen transmissibility, produce steep PO2 gradients within the wall, and give rise to oxygen-derived free radicals with ensuing tissue damage. Similarly, the reduction of molecular oxygen coupled to the oxidation of homocysteine may result in free radicals and hydrogen peroxide, which may damage endothelial cells. Oxidation of homocysteine may also modify LDL and enhance foam cell formation. Thus, a self-perpetuating mechanism may favor the progression of intimal thickening toward occlusive arterial disease, especially in the presence of hyperhomocyst(e)ineemia. Furthermore, there are autopsies data suggestive of an association between the extent of atherosclerosis in the cerebral and coronary circulations. In vivo studies also suggest that extracranial carotid artery atherosclerosis, measured by B-mode ultrasound, is associated with the extent of coronary atherosclerosis, measured by coronary angiography, as well as with the risk of acute myocardial infarction. Consequently, it is likely that our findings on carotid artery thickening also may have implications for coronary atherosclerosis.

High levels of homocyst(e)ine are lowered readily, in most patients, by the intake of 1–5 mg/day folic acid, occasionally requiring additional small amounts of vitamin B6, choline, or betaine; these supplements are inexpensive and usually innocuous in the absence of pernicious anemia.
Findings from the present cross-sectional analyses should be confirmed by examination of incidence data on atherosclerosis in the ARIC Study. Furthermore, whether normalization of hyperhomocyst(e)inemia will modify the course of arterial occlusive disease needs to be established by placebo-controlled clinical trials.

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