Evidence of Carotid Artery Wall Hypertrophy in Homozygous Homocystinuria

Jean-Louis Megnien, MD; Jérôme Gariepy, MD; Jean-Marie Saudubray, MD; Jean-Marc Nuoffer, MD; Nicolas Denarie, MD; Jaime Levenson, MD; Alain Simon, MD

Background—We aimed to determine whether intima-media thickness (IMT) was increased in the carotid artery of subjects with homocystinuria to better understand the in vivo contribution of homocysteine to early atherogenesis.

Methods and Results—We investigated ultrasonographically the right common carotid artery in 14 subjects with homozygous homocystinuria aged 3 to 34 years (mean, 13 years) and in 15 of their heterozygous parents aged 32 to 47 years (mean, 41 years) by comparison with 2 control groups of 15 healthy subjects of the same age. Far-wall IMT and lumen diameter were measured with a computerized program, and the cross-sectional area of the intima-media complex (CSA-IMC) was calculated from IMT and diameter. Comparison with their respective controls, adjusted for body surface area or height, showed that homozygotes had greater IMT (P<0.001) and CSA-IMC (P<0.05) and smaller diameter (P<0.05), whereas heterozygotes had values similar to their controls. Multivariate analysis of the arterial parameters with age, body surface area (or height), and plasma total homocysteine in the homozygous and heterozygous groups combined showed that IMT was related to age (P<0.05) and homocysteine (P<0.01), diameter was related to body surface area (P<0.001) or height (P<0.05), and CSA-IMC was related to age (P<0.05), body surface area (P<0.05) (but not height), and homocysteine (P<0.05).

Conclusions—Homozygous homocystinuria was associated with common carotid wall hypertrophy, whereas heterozygous disease was not. Such hypertrophy may reflect a smooth muscle proliferation induced by hyperhomocysteinemia and represent a promising target for testing vascular effects of therapeutic measures to lower homocysteine. (Circulation. 1998;98:2276-2281.)

Key Words: homocysteine • atherosclerosis • ultrasonics • carotid arteries

Homocysteine is a highly reactive amino acid. Its severe elevation, seen in homozygous homocystinuria, a rare disease due to inborn errors of homocysteine metabolism, is associated with premature cardiovascular complications induced by accelerated atherosclerosis and/or thromboembolism. The toxic effects of homocysteine on the vasculature have been well documented in experimental studies, consisting in particular of endothelial cell injury and smooth muscle cell proliferation, which represent 2 prominent features of atherogenesis. In contrast, the in vivo contribution of hyperhomocysteinemia to atherosclerosis in humans has been less fully investigated. One interesting way of investigation is to assess the effects of homocystinuria on markers of early atherosclerosis such as endothelial dysfunction or arterial wall thickening. Large-artery endothelial dysfunction has been reported in children with homozygous homocystinuria. In the present study, we hypothesized that homocystinuria might be associated with arterial wall hypertrophy because it has been shown experimentally that homocysteine at concentrations similar to those seen in homozygous homocystinuria has a direct growth-promoting effect on vascular smooth muscle cells. To this end, we used high-resolution B-mode ultrasonography to measure intima-media thickness (IMT) and lumen diameter in a large peripheral artery, the common carotid artery, of subjects with and without homozygous and heterozygous homocystinuria.

Methods

Study Subjects

Fourteen subjects with homozygous homocystinuria (10 cystathionine-β-synthase deficiencies and 4 defects of remethylation), including 2 pairs of siblings aged 3 to 34 years (mean, 13 years) and 15 of their heterozygous parents coming from 8 families and aged 32 to 47 years (mean, 41 years) underwent ultrasonographic investigation of the carotid artery between 1996 and 1997. They were compared with 2 control groups of healthy subjects of similar ages studied with the same technique of carotid investigation during the same period: (1) a control group for homozygotes including 7 males and 8 females aged 2 to 25 years (mean, 13 years) and (2) a control group for heterozygotes including 7 men and 8 women aged 32 to 49 years (mean, 41 years). The age at diagnosis of homozygous subjects ranged from 0 to 22 years (mean, 5 years).
TABLE 1. Characteristics of Subjects With Homocystinuria

<table>
<thead>
<tr>
<th></th>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>13 (9)</td>
<td>41 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>8/6</td>
<td>7/8</td>
<td>NS</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.49 (0.35)</td>
<td>1.69 (0.09)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.38 (0.57)</td>
<td>1.87 (0.29)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia, n</td>
<td>2</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, n</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking,* pack-years</td>
<td>1.4 (0–10)</td>
<td>7.5 (0–20)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Plasma total homocysteine,*</td>
<td>91 (9–179)</td>
<td>15 (4–56)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are number or mean, with SD or ranges in parentheses. Hypertension was defined as blood pressure >140 and/or >90 mm Hg; hypercholesterolemia, total cholesterol ≥6.2 mmol/L; and diabetes mellitus, blood glucose ≥7.7 mmol/L.

*Log-transformed comparison.

At the time of investigation, 12 homozygous subjects were consuming a low methionine diet, 3 were taking pyridoxine (of whom 2 were pyridoxine sensitive), 6 were taking folic acid supplements, 3 were taking cobalamin, and 11 were prescribed betaine. Three homozygous subjects had a previous history of cardiovascular disease, including 1 cerebral trunk thrombosis, 1 stroke with iliac artery stenosis, and 1 cerebral arterial spasm with iliac artery thrombosis. No homozygous subject had any ultrasonic evidence of arterial narrowing or plaque in the carotid arteries. Heterozygous subjects had no present or past history or sign of cardiovascular disease and did not take any cardiovascular drug treatment, but 1 subject had ultrasonic evidence of plaque in the left carotid artery bifurcation. The reasons that 9 heterozygous parents did not participate in the study were related to domestic or professional constraints and excluded any present or past history of cardiovascular disease. All homozygous and heterozygous subjects underwent evaluation of body size and traditional cardiovascular risk factors, including hypertension, hypercholesterolemia, diabetes mellitus, and smoking history estimated in pack-years (Table 1).

Arterial Investigations

A high-resolution ATL Ultramark 9 B-mode ultrasound system was used to measure IMT in the far wall of the right common carotid artery according to a standardized procedure previously described in detail. In brief, the IMT image, consisting of 2 parallel echogenic lumen-intima and media-adventitia interfaces visible on ≥1 cm of length of the vessel, was frozen in telediastole by ECG triggering and transferred to a computer for automated measurement by means of an edge-detection program (Iötec system, lódata), the principles and detailed description of which have been provided elsewhere. Simultaneously, the lumen diameter was imaged between the far-wall and near-wall lumen-intima interfaces (leading edges), frozen in telediastole, and transferred to the computer for automated measurement with the edge-detection program. The cross-sectional area of the intima-media complex (CSA-IMC) was calculated from IMT and lumen diameter (D) as π×IMT×(IMT+D).

Homocysteine Measurement

In all homozygous and heterozygous subjects, plasma total homocysteine was measured by a radioenzymatic assay by condensation of homocysteine in the presence of S-adenosyl-L-homocysteine hydrolase with C14-adenosine to form C14-adenosyl-homocysteine, which was then quantified. In the homozygous subjects, the value of homocysteine was the average of several measurements over time (2 to 9 measurements over 2 to 8 years), whereas in the heterozygous subjects, homocysteine was measured once at the time of investigation.

Statistical Analysis

Comparisons between groups were performed by use of Student’s t test for quantitative parameters and χ2 analysis for qualitative variables. Univariate regressions were assessed by linear regression analysis. Multiple regression analysis was used with arterial parameters as the dependent variables and age, body surface area (or height), and plasma total homocysteine as independent variables.

Results

In the homozygous group, plasma total homocysteine was elevated (>15 μmol/L) in 12 subjects but normal in the 2 pyridoxine-sensitive subjects. Compared with their control group, the homozygous group had greater IMT (P<0.001) and CSA-IMC (P<0.05) but similar lumen diameter; all these arterial parameters were derived from the common carotid (Figure 1, Table 2). After adjustment for height or body surface area, IMT and CSA-IMC remained greater in homozygotes than in controls (P<0.001 and P<0.05, respectively), whereas lumen diameter was smaller in homozygotes than in controls (P<0.05) (Table 2). In the homozygous group, positive univariate correlations existed between lumen diameter and age (r=0.80, P<0.001), height (r=0.85,
P<0.001), and body surface area (r=0.87, P<0.001) and between CSA-IMC and height (r=0.62, P<0.01) and body surface area (r=0.67, P<0.01). No correlation existed between plasma total homocysteine and any arterial parameter, even after adjustment for age and height or body surface area. Finally, IMT correlated negatively with age at diagnosis (r=0.54, P=0.05), whereas lumen diameter and CSA-IMC did not.

In the heterozygous subjects, plasma total homocysteine was normal in 13 subjects but elevated (≥15 μmol/L) in 2 subjects. Compared with their control group, the heterozygous group did not have different values of IMT, lumen diameter, and CSA-IMC (Figure 2, Table 2), and this lack of difference persisted after adjustment for height or body surface area (Table 2). In the heterozygous group, age, height, and body surface area did not correlate with any arterial parameter with the exception that body surface area correlated with diameter (r=0.61, P<0.05). IMT and CSA-IMC correlated positively and almost significantly with plasma total homocysteine (r=0.47, P=0.06 and r=0.48, P=0.07, respectively), whereas lumen diameter did not (r=0.18). After adjustment for age and body surface area, IMT remained almost significantly associated with homocysteine (P=0.06), whereas CSA-IMC did not.

In homozygous and heterozygous groups combined, multivariate analysis with arterial parameters as dependent variables and age, body surface area (or height), and plasma total homocysteine as independent variables showed the following: (1) IMT was positively related to age (P<0.05) and plasma total homocysteine (P<0.01); (2) lumen diameter was positively related to body surface area (P<0.001) (or height [P<0.05]); and (3) CSA-IMC was positively related to age (P<0.05), body surface area (P<0.05) (but not height), and plasma total homocysteine (P<0.05) (Table 3).

Discussion
The important new finding in this study is that compared with healthy children and young adults of their age, homozygotes

TABLE 2. Comparisons of Common Carotid Artery Dimensions Before and After Adjustment for Height or Body Surface Area Between Homozygotes, Heterozygotes, and Their Controls

<table>
<thead>
<tr>
<th></th>
<th>Homozygotes</th>
<th>Controls</th>
<th>Heterozygotes</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.50±0.01</td>
<td>0.44±0.01</td>
<td>0.50±0.01</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>Height-adjusted</td>
<td>0.50±0.01</td>
<td>0.44±0.01</td>
<td>0.50±0.01</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>BSA-adjusted</td>
<td>0.50±0.01†</td>
<td>0.44±0.01</td>
<td>0.50±0.01</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>5.01±0.14</td>
<td>5.30±0.14</td>
<td>5.59±0.17</td>
<td>5.93±0.18</td>
</tr>
<tr>
<td>Height-adjusted</td>
<td>5.02±0.09*</td>
<td>5.29±0.09</td>
<td>5.59±0.17</td>
<td>5.92±0.18</td>
</tr>
<tr>
<td>BSA-adjusted</td>
<td>5.00±0.09†</td>
<td>5.31±0.09</td>
<td>5.57±0.16</td>
<td>5.95±0.16</td>
</tr>
<tr>
<td>CSA-IMC, mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>8.70±0.27*</td>
<td>7.84±0.26</td>
<td>9.60±0.44</td>
<td>9.62±0.44</td>
</tr>
<tr>
<td>Height-adjusted</td>
<td>8.71±0.24*</td>
<td>7.83±0.23</td>
<td>9.60±0.44</td>
<td>9.62±0.44</td>
</tr>
<tr>
<td>BSA-adjusted</td>
<td>8.69±0.23*</td>
<td>7.85±0.22</td>
<td>9.57±0.41</td>
<td>9.65±0.41</td>
</tr>
</tbody>
</table>

BSA indicates body surface area.
*P<0.05; †P<0.001.
for homocystinuria had a clear-cut premature, large, artery-wall hypertrophy reflected by increased IMT and CSA-IMC. Such an increase persisted after adjustment for height or body surface area, which was performed because of the associations of carotid dimensions with height or body surface area found in our work, in agreement with a previous report. However, our result is in contrast to results of another study that showed that 12 homozygotes for homocystinuria (mean age, 24 years) were similar to healthy control subjects with regard to carotid artery IMT. Such a discrepancy might be due to important differences in the population age (11 years older than in the present study) and in the IMT measurement having been obtained from multiple sites (common carotid, bifurcation, and internal carotid) without the assistance of a computerized automated program of image analysis. Furthermore, the thickening process found in homozygotes in the present study existed despite appropriate medical management, perhaps because such management did not normalize homocysteine except in the 2 pyridoxine-sensitive subjects. This possibility is supported by the fact that the 2 homozygotes with pyridoxine sensitivity had values of IMT among the 3 lowest values of the homozygous group (full circles in Figure 1). We also observed a negative correlation of IMT with age at diagnosis. Because IMT did not correlate with age in the homozygous group, the correlation of IMT with age at diagnosis did not mean that older homozygotes had smaller IMTs. A possible explanation was that an older age at diagnosis might be evidence of a more benign clinical course of the disease with a less adverse effect on the arterial wall structure. Another interesting finding in homozygotes was a reduced common carotid artery lumen diameter compared with controls. Such a reduction in diameter reached statistical significance only after adjustment for height or body surface area because these 2 parameters were strongly and positively associated with lumen diameter, as previously noted by others. This original finding suggests that homozygous homocysteinemia could be associated with some degree of vasoconstriction of the common carotid artery.

By contrast, values of IMT, CSA-IMC, and lumen diameter were not different in heterozygote parents than in their controls. The lack of significant increase in IMT in heterozygotes is in agreement with a recent investigation that showed that the mean IMT values obtained in multiple carotid and femoral arterial segments were similar in 13 heterozygotes for cystathionine-β-synthase deficiency younger than 50 years of age and in 12 healthy controls. Therefore, our work indicates that any increased risk of atherosclerosis in heterozygotes may not be reflected by early structural change of the common carotid artery walls, at least in the absence of hyperhomocysteinemia, as in the majority of our heterozygous subjects. It may also be that the heterozygotes were too young to have developed structural vascular changes. Nevertheless, because our B-mode measurements were obtained from only the proximal common carotid artery, it is possible that there might be demonstrable IMT differences with regard to the bifurcation or the internal carotid artery, which were not studied in the present investigation. In addition, our results in heterozygotes show some discrepancy compared with results from several previous studies. Both the ARIC and the Framingham studies showed associations of elevated homocysteine with carotid wall thickening, albeit in older populations and with the use of quite different methodology, such as the incorporation of intrusive plaque in IMT measurement and IMT measurement at multiple carotid arterial segments or at the carotid bifurcation. Two other studies showed that an early sign of premature arterial disease in the carotid and femoral arteries was present in heterozygotes for cystathionine-β-synthase deficiency and that premature arterial occlusive disease was associated with a higher prevalence of moderate hyperhomocysteinemia, but the characteristics of these subjects and the arterial methodology of these studies were too different from the current work to allow a pertinent comparison to be made.

The mechanisms by which homocystinuria, in its homozygous trait, predisposes to atherosclerosis might be related to abnormal vascular growth. Indeed, homozygous subjects exhibited an elevation not only of IMT but also of CSA-IMC.

<table>
<thead>
<tr>
<th>Model</th>
<th>IMT</th>
<th>β (SE)</th>
<th>P</th>
<th>Diameter</th>
<th>β (SE)</th>
<th>P</th>
<th>CSA-IMC</th>
<th>β (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Age</td>
<td>0.002 (0.001)</td>
<td>0.036</td>
<td>0.009 (0.011)</td>
<td>0.41</td>
<td>0.053 (0.024)</td>
<td>0.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body surface area</td>
<td>−0.014 (0.019)</td>
<td>0.46</td>
<td>0.905 (0.239)</td>
<td>&lt;0.001</td>
<td>1.122 (0.537)</td>
<td>0.047</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma total homocysteine*</td>
<td>0.025 (0.009)</td>
<td>0.008</td>
<td>0.026 (0.111)</td>
<td>0.82</td>
<td>0.579 (0.251)</td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>25%</td>
<td>61%</td>
<td>48%</td>
<td>27%</td>
<td>49%</td>
<td>42%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Log transformed.

Log (SE) indicates regression coefficient (standard error).
a parameter that incorporates in its calculation both IMT and lumen diameter and which is therefore more appropriate to describe arterial mass than IMT alone.\textsuperscript{11} The increased common carotid arterial mass observed in homozygous patients is consistent with the recent in vitro demonstration that homocysteine at concentrations similar to those seen in homoyzogotes enhances smooth muscle proliferation.\textsuperscript{7} This phenomenon is an important component of medial and intimal hypertrophy, which can both participate in the thickening process of the carotid wall.\textsuperscript{10,11} Nevertheless in the present study, the role of elevated homocysteine in carotid arterial wall hypertrophy was not obvious in separate analysis of the homozygous and heterozygous groups. In the homozygous group, plasma total homocysteine did not correlate with IMT and CSA-IMC, possibly because of a confounding effect of therapy. In the heterozygous group, the correlation of IMT and CSA-IMC with plasma total homocysteine almost attained statistical significance, even after adjustment for age and body surface area (or height) in the case of IMT. One can speculate that these correlations, observed with fasting plasma homocysteine values that were within the normal ranges except in 2 subjects, would have been closer if the heterozygous patients had been further characterized with a methionine-loading test.\textsuperscript{19} However, the methionine-loading test is a cumbersome procedure that was judged impracticable in the investigation of our heterozygous volunteers. By contrast, the influence of homocysteine on carotid wall hypertrophy was better evidenced by multivariate analysis of carotid artery dimensions with plasma total homocysteine, age, and body surface area as independent variables (model 1) or homocysteine, age, and height as independent variables (model 2) in the homozygous and heterozygous groups combined. Such analyses showed that IMT and CSA-IMC were related to homocysteine and age in both models, whereas lumen diameter was related only to body surface area in model 1 or to height in model 2. These findings suggest that hyperhomocysteinemia can be a strong risk factor for arterial wall hypertrophy, as suggested in a few recent epidemiological studies.\textsuperscript{15,20} However, the independence of its association with IMT and CSA-IMC should be considered with caution in the current work because the multivariate analysis was limited by the small number of subjects and by restricting attention to only homocystinuric patients.

The small number of subjects was a study limitation that merits careful consideration, as does the fact that the B-mode measurements were obtained only from the proximal common carotid artery. The small number of homozygotes and, consequently, of their obligate heterozygote parents is due to the extreme rarity of the homozygote trait. In addition to the possibility that the multivariate analysis will be compromised with regard to the independence of the association between homocysteine and IMT, this small number of patients may be responsible for a type II error when IMT is compared between heterozygotes and controls. Because the observed IMT difference between heterozygotes and controls is quite small (0.026 mm), the probability of a type II error is high. On the other hand, this IMT difference is too small, compared with the greater IMT differences previously found with the same technique between hypertensive or hypercholesterolemic adults and controls (0.05 to 0.10 mm),\textsuperscript{10,21} to have a real physiopathological relevance. B-mode measurement of arterial dimensions in the proximal common carotid artery precludes the possibility of obtaining information from the bifurcation or the internal carotid artery, where turbulent flow may predispose to effects related to both thrombosis and atherosclerosis and therefore may induce the greatest effects of homocysteine on arterial structure. Nevertheless, the limitation due to the focus of attention on the common carotid artery may be put into perspective by considerations of methodological order. First, IMT measurement is the most accurate in the common carotid artery because this vessel is straight, superficial, and almost parallel to the skin.\textsuperscript{22} Such geometric conditions are not met by the bifurcation and the internal carotid artery, thus explaining why IMT measurement in these arterial segments has 2 or 3 times greater variability than in the common carotid artery.\textsuperscript{22} This greater variability would increase considerably the number of patients required to demonstrate a difference in IMT even if one would expect the greatest difference in the internal carotid or in the bifurcation. Second, the accuracy of the CSA-IMC calculation is based on the assumption that IMT has approximately the same value along the artery circumference.\textsuperscript{11} Such an assumption can be accepted in the proximal common carotid artery, which is a cylindrical tube with a laminar flow inside its lumen. In contrast, it is not valid in the bifurcation or in the internal carotid artery. These arterial segments do not have a cylindrical geometry, and they present local variations in flow and turbulence capable of inducing different IMT values along the artery circumference, which does not permit the calculation of CSA-IMC by use of a cylindrical model. Finally, measurement of lumen diameter only in the common carotid artery may constitute a limitation of greater importance, because previous investigation has identified important differences between the association of risk factors in the common carotid as opposed to the internal carotid artery.\textsuperscript{23} Such a difference may exist regarding the effect of homocysteine on lumen diameter. Therefore, in the future, scanning procedures specifically aimed at diameter measurement in various carotid artery segments should be performed in patients with homocystinuria.

In conclusion, because arterial wall thickening assessed in the carotid artery reflects generalized changes elsewhere in the arterial tree\textsuperscript{24,25} and may be related to subsequent vascular events,\textsuperscript{26,27} our work suggests that this early arterial alteration is a potential method by which hyperhomocysteinemia may predispose to vascular disease. It is also suggested that more aggressive therapy may be required in homozygous homocystinuria to retard or even reverse arterial wall hypertrophy and that further clinical trials are needed to demonstrate this point.

Acknowledgments

This study is supported in part by the CRI-INSERM No. 4U010B. We thank Maria Crichi for her excellent secretarial assistance. We gratefully acknowledge collaboration with Pr Pierre Kamoun and Bernadette Chadeaux, Department of Biochemistry, Necker Hospital, for measuring plasma total homocysteine.
References

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Circulation. 1998;98:2276-2281
doi: 10.1161/01.CIR.98.21.2276
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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