Carotid and Femoral Artery Wall Thickness and Stiffness in Patients at Risk for Cardiovascular Disease, With Special Emphasis on Hyperhomocysteinemia

Tineke J. Smilde, Franchette W.P.J. van den Berkmortel, Godfried H.J. Boers, Hub Wollersheim, Theo de Boo, Herman van Langen, Anton F.H. Stalenhoef

Abstract—Recent developments in ultrasound technology enable the noninvasive measurement of structural and functional vessel wall changes. Until now, the effect of homocysteine on the arterial wall has remained unclear: reports on intima-media thickness (IMT) yield conflicting results, whereas data on vessel wall stiffness are lacking. Because several cardiovascular risk factors result in an increased IMT or stiffness, different groups at risk for atherosclerotic disease, with special emphasis on hyperhomocysteinemia, were studied. Nineteen patients homozygous and 14 subjects heterozygous for cystathionine β-synthase (CBS) deficiency, 21 patients with familial hypercholesterolemia (FH), 15 patients with essential hypertension, 20 smokers, and 28 control subjects were studied. The IMT values (both right and left) of the common carotid artery (CCA), bulb (BUL), internal carotid artery (ICA), and common femoral artery (CFA) were measured in millimeters by high-resolution ultrasound (Biosound). The distensibility (DC, in \(10^{-3} \cdot \text{kPa}^{-1}\)) and compliance (CC in \(\text{mm}^2 \cdot \text{kPa}^{-1}\)) coefficients of the CCA (right and left) and CFA (right) were determined by a wall track system (Pie Medical). The mean IMT of the posterior wall in the CCA was 0.70±0.09 mm in healthy controls. For patients with vascular disease, FH, and hypertension and in smokers, the mean CCA IMT was larger, whereas no major differences in IMT were observed in patients either homozygous or heterozygous for CBS deficiency. The DC and CC in the right CCA were 23.5±6.9 (\(10^{-3} \cdot \text{kPa}^{-1}\)) and 0.9±0.3 (\(\text{mm}^2 \cdot \text{kPa}^{-1}\)) in healthy subjects, slightly lower in patients homozygous for CBS deficiency, and clearly lower in patients with vascular disease, FH, and hypertension. No positive correlation was found between plasma homocysteine level and either IMT, CC, or DC. Because smoking was a confounder in each risk group, a stepwise regression analysis was carried out to assess the contribution of each risk factor on IMT and arterial wall stiffness. Age explained most of the variation in IMT of the CCA (coefficient of determination \(R^2\) of 0.34), whereas \(R^2\) values for serum low density lipoprotein cholesterol, smoking (pack-years), and systolic blood pressure were 0.08, 0.07, and 0.06, respectively. Homocysteine did not contribute to variation in IMT in both the CCA and CFA. Age and smoking contributed to the variation in IMT in the CFA. The variation in DC and CC in the right CCA and right CFA could in part be explained by age, low density lipoprotein cholesterol, and blood pressure. Plasma homocysteine concentration explained only a small proportion of the variation in DC in the CCA (\(R^2=0.02\)) and in CC in the CFA (\(R^2=0.04\)). In this study, no relationship was found between homocysteine level and the thickness of the arterial wall, with only a marginal influence on stiffness. (Arterioscler Thromb Vasc Biol. 1998;18:1958-1963.)

Key Words: intima-media thickness ■ artery wall stiffness ■ cardiovascular risk factors ■ hyperhomocysteinemia

Established risk factors for atherosclerotic disease are hypercholesterolemia, hypertension, and smoking. In the last 10 years, hyperhomocysteinemia has also been recognized as a risk factor for thromboembolism and atherosclerosis.1-4 Arterial intima-media thickness (IMT) and functional vessel wall properties, like distensibility and compliance, of superficial arteries (carotid and femoral)5-21 can be evaluated by high-resolution B- and M-mode ultrasound and are associated with the presence of atherosclerotic disease elsewhere.22-25 Because various risk factors may affect the arterial wall to a different extent, it is of interest to explore whether the changes can be measured by these noninvasive ultrasound techniques in patients with different cardiovascular risk factors to monitor either progression or regression of vessel wall damage.

The mechanism by which homocysteine exerts its effects on the arterial wall is still unclear. Until now, reports on an association of hyperhomocysteinemia with increased IMT have yielded conflicting results.12-16 Furthermore, no ultrasound data are available on the effect of elevated levels of homocysteine on functional vessel wall properties. Several studies indicate that even mild hypercholesterolemia is associated with intima-media thickening, which may be reduced...
by cholesterol lowering.17–19 Little is known about the influence of hypercholesterolemia on arterial stiffness in the carotid and femoral artery.20 Patients with hypertension demonstrate increases in IMT,21 and even in borderline hypertensive patients with hyperhomocysteinemia were investigated. All received vitamin supplements (vitamin B<sub>6</sub>, folic acid, and vitamin B<sub>12</sub>). In addition, 21 patients with familial hypercholesterolemia characterized by elevated plasma LDL cholesterol levels and the presence of tendon xanthomas were studied. They participated in a cholesterol-lowering trial and were in the placebo run-in phase by the time measurements were performed. Furthermore, 20 subjects who had smoked for at least 20 pack-years (1 pack-year was defined as smoking 20 cigarettes a day for a period of 1 year), 15 patients with untreated essential hypertension (systolic blood pressure >160 mm Hg and diastolic blood pressure >95 mm Hg, measured on 3 separate occasions after 5 minutes of supine rest), and 28 nonsmoking healthy controls were studied. In all participants, special attention was given to the presence of cardiovascular disease, medication, smoking behavior, and family medical history. Patients with diabetes or impaired renal function were excluded. None of the participants used antihypertensive drugs. After an overnight fast, serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and homocysteine levels were measured. The study was approved by the Ethics Committee of our hospital, and informed consent was obtained from each individual.

**Methods**

In a cross-sectional design, 19 patients with homocystinuria due to homozygous cystathionine β-synthase (CBS) deficiency, 14 of their heterozygous relatives, and 15 patients with premature vascular disease (coronary heart, cerebral vascular, and/or peripheral vascular disease before the age of 50 years) in the presence of mild hyperhomocysteinemia were investigated. All received vitamin supplements (vitamin B<sub>6</sub>, folic acid, and vitamin B<sub>12</sub>). In addition, 21 patients with familial hypercholesterolemia characterized by elevated plasma LDL cholesterol levels and the presence of tendon xanthomas were studied. They participated in a cholesterol-lowering trial and were in the placebo run-in phase by the time measurements were performed. Furthermore, 20 subjects who had smoked for at least 20 pack-years (1 pack-year was defined as smoking 20 cigarettes a day for a period of 1 year), 15 patients with untreated essential hypertension (systolic blood pressure >160 mm Hg and diastolic blood pressure >95 mm Hg, measured on 3 separate occasions after 5 minutes of supine rest), and 28 nonsmoking healthy controls were studied. In all participants, special attention was given to the presence of cardiovascular disease, medication, smoking behavior, and family medical history. Patients with diabetes or impaired renal function were excluded. None of the participants used antihypertensive drugs. After an overnight fast, serum total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and homocysteine levels were measured. The study was approved by the Ethics Committee of our hospital, and informed consent was obtained from each individual.

**Homocysteine and Cholesterol Determination**

An EDTA-blood sample for determination of the fasting homocysteine concentration was obtained and centrifuged for 10 minutes at 1800g, and the plasma was stored at approx. 30°C until analysis. In all hyperhomocysteinemic subjects, both the homocysteine determination for CBS deficiency as well as the vascular disease patients with mild hyperhomocysteinemia, an oral methionine load homocysteine level was obtained. During this procedure, an oral dose of L-methionine (0.1 g/kg body weight) was administered in orange juice. Six hours after the methionine load, a second EDTA-plasma sample was drawn for postload homocysteine concentration. Total (free plus bound) homocysteine levels were measured using tri-n-butyolphosphate as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate as the fluorochromophore, followed by high-performance liquid chromatography with fluorescence detection.24 Hyperhomocysteinemia was defined as elevated fasting homocysteine levels (>18 μmol/L) and/or a postmethionine load homocysteine level >97.5th percentile in healthy controls, ie, 51 μmol/L for females and 54 μmol/L for males.25 Plasma cholesterol and triglycerides were determined by commercially available enzymatic methods (Boehringer Mannheim No. 237574 and Sera-Pak, Miles No. 6639, respectively); to determine HDL cholesterol, the PEG 6000 precipitation method was used.26 LDL cholesterol was calculated by the Friedewald formula. Normal values of serum total cholesterol were defined as 4.7 to 6.5 mmol/L (divide by 0.026 for mg/dL); of HDL cholesterol, 1.10 to 1.70 mmol/L for females and 0.95 to 1.50 mmol/L for males; of LDL cholesterol, <4.5 mmol/L; and of triglycerides, 0.8 to 2.0 mmol/L (divide by 0.011 for mg/dL).

**Ultrasound Imaging**

The ultrasound examinations were performed using a Biosound phase-2 real-time scanner (Biosound Esote) equipped with a 10-MHz transducer, 2 monitors for displaying the B-mode ultrasound images, and a spectrum analyzer for the Doppler signals. Three 10-mm segments were scanned bilaterally: the distal portion of the common carotid artery (CCA), the carotid bulb (BUL), and the proximal portion of the internal carotid artery (ICA). Images were “grabbed” by a computer, stored on optical disk, and analyzed with a semiautomatic software program (Eureqa, TSA Co).27 IMT measurements were performed on both anterior and posterior walls of the CCA and BUL, and on the posterior wall of the ICA and right side of the common femoral artery (CFA). Intraobserver and interobserver coefficients of variation were <5%.28

**Distensibility and Compliance**

The vessel wall movement-detector system has been described in detail by Hoeks et al.29 The system used consisted of a wall track system (Pie Medical) and a data acquisition system connected to a personal computer. With a 7.5-MHz transducer, a 2-dimensional B-mode image was made from the CCA, 2 cm proximal to the BUL. An M-mode line perpendicular to the vessel was selected. After switching to M-mode, storage of data was started during 3 to 5 cardiac cycles. The RF signals during this period were digitized and temporarily stored. For both the anterior and posterior wall segments, the cumulative change in phase between successive RF lines was calculated. The difference between the anterior and posterior wall represents arterial distension, which is the change in diameter during 1 heart cycle. Data for arterial diastolic diameter (D) and distension (ΔD) were obtained for each heart beat. Brachial blood pressure was measured every 3 minutes with a semiautomatic oscillometric device (Dinamap). The mean of the total blood pressure recordings during 40 minutes was taken. Pulse pressure was defined as systolic minus diastolic blood pressure (ΔP). Vessel wall properties were calculated according to the following equations:

1. **Distensibility Coefficient (DC) = (2ΔD/D)ΔP** (in 10<sup>−3</sup> · kPa<sup>−1</sup>)

2. **Compliance Coefficient (CC) = rΔD/D(2ΔP)** (in mm<sup>2</sup> · kPa<sup>−1</sup>)

**Statistical Analysis**

The Pearson correlation was used to assess the univariate association between different risk factors and IMT, DC, and CC in different segments of the carotid and femoral artery and the correlation between DC and IMT and CC and IMT. To determine which factor explained a given dependent variable, a stepwise regression procedure was carried out. Dependent variables were IMT, DC, and CC. Independent variables were age, sex, body mass index, blood pressure, smoking (pack-years), and LDL cholesterol, HDL cholesterol, triglyceride and homocysteine levels. In addition, a regression analysis was performed in which the interactions (cross products) of smoking with each of the above-mentioned independent variables were added as independent variables. Statistical analyses were performed using SAS (version 6.12, SAS Institute Inc).

**Results**

Clinical and biochemical characteristics of the study groups are summarized in Table 1. The IMT of the posterior and anterior walls of the different groups are given in Table 2 and functional vessel wall properties are presented in Table 3. In healthy controls, the IMT of the posterior wall of the CCA was 0.70 ± 0.09 mm. In patients with premature vascular disease, FH, and hypertension in smokers, the IMT was larger, especially when the transducer was moved cranially along the carotid artery. The IMT of the posterior wall in the BUL was 0.88 ± 0.25...
TABLE 1. Clinical and Biochemical Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>HH</th>
<th>HZ</th>
<th>V</th>
<th>FH</th>
<th>H</th>
<th>S</th>
</tr>
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<tbody>
<tr>
<td>Age, y</td>
<td>39±11</td>
<td>36±13</td>
<td>44±18</td>
<td>48±4</td>
<td>46±11</td>
<td>44±13</td>
<td>42±8</td>
</tr>
<tr>
<td>Body mass index, kg · m⁻²</td>
<td>24.0±1.6</td>
<td>24.0±3.8</td>
<td>25.1±5.5</td>
<td>25.7±3.4</td>
<td>24.6±1.6</td>
<td>25.6±5.2</td>
<td>24.1±2.0</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>127.8±8.1</td>
<td>131.7±12.3</td>
<td>129.2±16.7</td>
<td>135.8±13.8</td>
<td>128.7±11.6</td>
<td>187.5±19.9</td>
<td>133.0±9.6</td>
</tr>
<tr>
<td>Diastolic</td>
<td>76.2±8.6</td>
<td>81.5±9.8</td>
<td>74.1±12.6</td>
<td>78.8±9.0</td>
<td>71.9±7.2</td>
<td>105.3±14.5</td>
<td>77.8±6.5</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>0</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>12</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Cardiovascular disease, n</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>14</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>4.9±0.7</td>
<td>5.0±1.1</td>
<td>5.6±1.1</td>
<td>5.9±0.9</td>
<td>11.9±2.2</td>
<td>5.2±0.8</td>
<td>5.4±0.9</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.1±0.4</td>
<td>1.1±0.7</td>
<td>1.7±1.0</td>
<td>2.3±1.5</td>
<td>1.7±0.6</td>
<td>1.4±0.7</td>
<td>1.7±1.0</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.2±0.2</td>
<td>1.2±0.2</td>
<td>1.1±0.4</td>
<td>1.2±0.4</td>
<td>1.2±0.2</td>
<td>0.9±0.1</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.3±0.6</td>
<td>3.3±0.9</td>
<td>3.8±1.1</td>
<td>3.8±0.8</td>
<td>9.8±2.1</td>
<td>3.7±0.8</td>
<td>3.8±0.9</td>
</tr>
<tr>
<td>Homocysteine, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>10.6±1.8</td>
<td>62.5±43.6</td>
<td>11.2±1.3</td>
<td>14.4±5.8</td>
<td>9.6±1.4</td>
<td>11.2±1.6</td>
<td>10.7±1.5</td>
</tr>
<tr>
<td>6 h After methionine loading</td>
<td>...</td>
<td>234.7±83.3</td>
<td>39.5±7.5</td>
<td>60.8±20.5</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

C indicates healthy control subjects; HH, homozygous for CBS deficiency; HZ, heterozygous for CBS deficiency; V, patients with vascular disease and mild hyperhomocysteinemia; H, patients with hypertension; and S, smokers. To convert cholesterol measurements to mg/dL, divide by 0.026 mg/dL; for triglycerides conversion, divide by 0.011 mg/dL. Results are mean±SD.

in healthy controls and 1.31±0.45, 1.25±0.35, 1.36±0.36, and 1.23±0.37 mm in patients with premature vascular disease, patients with FH, hypertensives, and smokers, respectively. Pearson correlation coefficients between different risk factors and dependent variables such as IMT, DC, and CC are given in Table 4. The levels of serum LDL cholesterol, blood pressure, body mass index, and smoking were positively correlated with the IMT in the carotid artery, whereas smoking and LDL cholesterol were positively correlated with the IMT of the CFA (Table 4). A negative correlation was found between homocysteine and IMT in the CCA. The results of the stepwise regression procedure are given in Table 5. Age, serum LDL cholesterol, smoking, and systolic blood pressure explained partially the variation in arterial wall thickness. The effect of LDL cholesterol was especially important in the ICA, where 17% of the variation in IMT could be explained by LDL cholesterol. When smoking and elevated LDL cholesterol were present at the same time, the effect on IMT in the ICA increased to almost 30%. For the mean IMT in the carotid artery, ie, after combining the measurements of the posterior and anterior walls of the CCA and BUL and the posterior wall of the ICA, the contribution of LDL cholesterol alone on IMT was 9% but increased to 30% whenever patients smoked. Homocysteine showed no relationship with IMT, and there was no additional effect of homocysteine on the effect of smoking on IMT.

The DC and CC of the right CCA were 23.5±6.9 10⁻³·kPa⁻¹ and 0.9±0.3 mm²·kPa⁻¹ in healthy controls and tended to be lower in patients at risk. The levels of serum LDL cholesterol, blood pressure, and body mass index were negatively correlated with CC and DC in both the CCA and CFA. Smoking was negatively correlated with the DC in the right CCA only. There was a strong, negative correlation between IMT and the DC and the CC in the CCA and CFA. No negative correlation was found between functional vessel wall properties and homocysteine levels. Age, serum LDL cholesterol, and systolic blood pressure partially explained the variation in DC. Plasma homocysteine contributed to a small proportion of the variation in the DC of the CCA and in

TABLE 2. IMT in the Different Study Groups

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>HH</th>
<th>HZ</th>
<th>V</th>
<th>FH</th>
<th>H</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA posterior wall</td>
<td>0.70±0.09</td>
<td>0.71±0.14</td>
<td>0.81±0.19</td>
<td>0.92±0.15</td>
<td>0.98±0.29</td>
<td>1.00±0.21</td>
<td>0.90±0.19</td>
</tr>
<tr>
<td>CCA anterior wall</td>
<td>0.75±0.11</td>
<td>0.80±0.28</td>
<td>0.83±0.23</td>
<td>1.00±0.29</td>
<td>0.99±0.21</td>
<td>1.03±0.19</td>
<td>0.93±0.19</td>
</tr>
<tr>
<td>BUL posterior wall</td>
<td>0.88±0.25</td>
<td>1.04±0.57</td>
<td>1.03±0.41</td>
<td>1.31±0.45</td>
<td>1.25±0.35</td>
<td>1.36±0.36</td>
<td>1.23±0.37</td>
</tr>
<tr>
<td>BUL anterior wall</td>
<td>0.84±0.12</td>
<td>0.79±0.19</td>
<td>1.04±0.42</td>
<td>1.11±0.29</td>
<td>1.14±0.22</td>
<td>1.49±0.38</td>
<td>1.23±0.28</td>
</tr>
<tr>
<td>ICA posterior wall</td>
<td>0.66±0.18</td>
<td>0.64±0.15</td>
<td>0.66±0.26</td>
<td>1.15±0.68</td>
<td>1.08±0.53</td>
<td>0.92±0.31</td>
<td>0.98±0.34</td>
</tr>
<tr>
<td>CFA posterior wall</td>
<td>0.74±0.23</td>
<td>1.25±1.11</td>
<td>1.07±0.36</td>
<td>0.92±0.15</td>
<td>1.60±0.72</td>
<td>1.29±0.62</td>
<td>1.49±0.79</td>
</tr>
</tbody>
</table>

See the footnote to Table 1 for explanation of abbreviations. Posterior and anterior wall measurements were performed in the CCA and BUL and posterior wall measurements in the ICA. Data are presented as the mean of both left and right sides. Posterior wall measurements of the right CFA are shown. Data are mean±SD in mm. Success percentage: 100% of posterior and anterior wall measurements were obtained in the CCA, 80% of posterior wall measurements in the BUL, and 60% of anterior wall measurements in the BUL. The ICA could be assessed in 85% of all subjects.
the CC of the CFA (Table 5). There was only a slight increase in the effect of homocysteine on the CC of the CFA whenever subjects smoked (from 4% to 7%). The diameters of both the CCA and CFA were explained by age and sex only, 9% and 10%, respectively, in the CCA and 5% and 23%, respectively, in the CFA. Homocysteine did not contribute to the variation in systolic diameter.

Discussion

LDL cholesterol, systolic blood pressure, and smoking are related to the IMT in the carotid artery. The effect of serum cholesterol on arterial wall thickness is well known, and measuring IMT in intervention trials is an important tool to estimate the progression or regression of early atherosclerosis. In the current study, the contribution of LDL cholesterol to IMT was more pronounced in the ICA, especially whenever patients smoked. Therefore, it is important to consider local changes and more general changes in IMT separately, especially in patients with increased LDL cholesterol. Higher LDL cholesterol levels result in stiffer arteries, although the effect on DC and CC was less pronounced than on IMT.

Increases in IMT and the stiffness of carotid arteries have been demonstrated in patients with hypertension, but it is still not known whether this stiffness is a consequence of an elevated distending pressure or the result of intima-media thickening in the vessel wall. Smoking contributed to IMT thickening but did not explain the variation in the DC. It is well known that smoking even 1 cigarette causes a short-term increase in arterial wall stiffness, whereas no obvious long-term effect of smoking on arterial stiffness has been observed.

Plasma homocysteine was not related to IMT in both the CCA and CFA, despite a marked increase in homocysteine in patients homozgyous for CBS deficiency. Vascular disease patients were selected because of the presence of early-onset disease (ie, before the age of 50 years) and the presence of mild hyperhomocysteinemia; unfortunately, all vascular disease patients smoked, thereby disturbing an independent analysis of the effects of vascular disease alone versus those of smoking alone. In our study, homocysteine did not add to the effect of smoking on arterial wall thickness. However, homocysteine contributed to a minor proportion of the variation in the DC in the CCA and in the CC in the CFA on the

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### TABLE 4. Pearson Correlations Between IMT Variables of the CCA, ICA, and CFA; DC and CC of the CCA and CFA; and Age, Body Mass Index (BMI), Mean Arterial Pressure (MAP), Smoking, Total Cholesterol (TC), Triglycerides (TG), LDL Cholesterol (LDL-C), HDL Cholesterol (HDL-C), and Homocysteine. Correlations Between IMT and DC and CC Are Also Indicated

<table>
<thead>
<tr>
<th>IMT</th>
<th>Age</th>
<th>BMI</th>
<th>MAP</th>
<th>Smoking</th>
<th>TC</th>
<th>TG</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Homocysteine</th>
<th>IMT CCA</th>
<th>IMT CFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT CCA</td>
<td>0.56†</td>
<td>0.29‡</td>
<td>0.34*</td>
<td>0.37*</td>
<td>0.41*</td>
<td>0.21‡</td>
<td>0.39*</td>
<td>-0.21‡</td>
<td>-0.22‡</td>
<td>...</td>
<td>0.27†</td>
</tr>
<tr>
<td>IMT BUL</td>
<td>0.49*</td>
<td>0.19‡</td>
<td>0.29‡</td>
<td>0.35*</td>
<td>0.25‡</td>
<td>0.27†</td>
<td>0.24‡</td>
<td>-0.15</td>
<td>-0.18</td>
<td>0.39*</td>
<td>0.57*</td>
</tr>
<tr>
<td>IMT ICA</td>
<td>0.43*</td>
<td>0.11</td>
<td>0.16</td>
<td>0.42*</td>
<td>0.40*</td>
<td>0.20‡</td>
<td>0.42*</td>
<td>-0.13</td>
<td>-0.13</td>
<td>0.50*</td>
<td>0.43*</td>
</tr>
<tr>
<td>IMT CFA</td>
<td>0.39*</td>
<td>0.19‡</td>
<td>0.11</td>
<td>0.40*</td>
<td>0.22‡</td>
<td>0.15‡</td>
<td>0.23‡</td>
<td>0.07</td>
<td>-0.13</td>
<td>0.27‡</td>
<td>...</td>
</tr>
<tr>
<td>DC (right) CCA</td>
<td>-0.60*</td>
<td>-0.27†</td>
<td>-0.51*</td>
<td>-0.25‡</td>
<td>-0.29‡</td>
<td>-0.16*</td>
<td>-0.27†</td>
<td>0.009</td>
<td>0.03</td>
<td>-0.45*</td>
<td>-0.36*</td>
</tr>
<tr>
<td>CC (right) CCA</td>
<td>-0.42*</td>
<td>-0.20‡</td>
<td>-0.41*</td>
<td>-0.10</td>
<td>-0.19‡</td>
<td>-0.13*</td>
<td>-0.17</td>
<td>-0.03</td>
<td>-0.00</td>
<td>-0.25†</td>
<td>-0.29‡</td>
</tr>
<tr>
<td>Diameter CCA</td>
<td>0.33*</td>
<td>0.13</td>
<td>0.15</td>
<td>0.21†</td>
<td>0.20†</td>
<td>0.04‡</td>
<td>-0.18</td>
<td>0.05</td>
<td>-0.07</td>
<td>0.45*</td>
<td>0.12</td>
</tr>
<tr>
<td>DC (right) CFA</td>
<td>-0.40*</td>
<td>-0.34‡</td>
<td>-0.36*</td>
<td>-0.16</td>
<td>-0.21‡</td>
<td>-0.18*</td>
<td>-0.19‡</td>
<td>-0.03</td>
<td>0.00</td>
<td>-0.28†</td>
<td>-0.34‡</td>
</tr>
<tr>
<td>CC (right) CFA</td>
<td>-0.38*</td>
<td>-0.29†</td>
<td>-0.37§</td>
<td>-0.13</td>
<td>-0.30‡</td>
<td>-0.26*</td>
<td>-0.28†</td>
<td>-0.05</td>
<td>-0.03</td>
<td>-0.31§</td>
<td>-0.32§</td>
</tr>
<tr>
<td>Diameter CFA</td>
<td>0.34*</td>
<td>0.02</td>
<td>0.15</td>
<td>-0.08</td>
<td>-0.16</td>
<td>-0.11</td>
<td>-0.15</td>
<td>-0.03</td>
<td>-0.015</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*P<0.001, †P<0.01, ‡P<0.05, §P>0.05.
right side, and this contribution increased whenever patients smoked. Age, LDL cholesterol, and systolic blood pressure explained most of the observed variation in the DC.

In previous reports, data on the effect of homocysteine on arterial wall thickness are conflicting. Clarke et al reported that hyperhomocysteinemia is a weak risk factor for asymptomatic extracranial carotid atherosclerosis. The mean IMT of the posterior wall of the CCA between heterozygous CBS subjects and controls was not statistically different. In 1993, Malinow et al published a study of 310 case-control pairs. Subjects without a history of occlusive arterial disease were selected as cases when at least 2 unilateral measurements of the carotid artery (CCA, BUL, and ICA) showed an increased IMT >1.6 mm in the CCA. Paired controls were selected among persons without evidence of carotid thickening. In that study, levels of homocysteine were positively correlated with carotid arterial wall thickness. Nevertheless, this positive correlation was no longer significant after adjustment for age, waist-hip ratio, serum cholesterol, smoking, and blood pressure. Rubba et al found no differences between IMT in patients homozygous for homocystinuria compared with controls. This result is in accordance with that of de Valk et al, who also reported no difference in IMT between heterozygous relatives of patients with homozgyous homocystinuria and controls. Tonstad et al found a positive relation between homocysteine level and IMT in children with FH and controls. No subject had homocysteine levels >14.1 μmol/L. In a study of elderly subjects, homocysteine was associated with isolated systolic hypertension. In this population of older subjects, homocysteine was related to atherosclerosis only among normotensive individuals. Duplex scanning of the carotid artery was performed to assess the amount of stenosis, but IMT was not measured. Aronow et al demonstrated that high plasma homocysteine levels and low plasma folate and vitamin B12 levels were associated with a higher prevalence of extracranial carotid arterial disease in a cohort of elderly women. Selhub et al found that nonfasting plasma homocysteine concentrations were associated with extracranial carotid artery stenosis in a population-based cohort of elderly people with a mean age of 75 years. In this study, the risk of stenosis ≥25% was increased even in subjects with homocysteine levels considered normal or slightly elevated. It is possible that the stenosis measured in the studies of Selhub, Aronow, and Sutton-Tyrrell and their coworkers was not specific arterial wall thickening but the result of arterial thrombosis.

Although our groups are relatively small and smoking is a major confounder, it is remarkable that arterial wall thickening could not be explained by homocysteine level, despite the almost 10-fold elevated in patients homozygous for CBS deficiency. In contrast, LDL cholesterol, blood pressure, and smoking all contributed to IMT. The effect of LDL cholesterol was stronger when the transducer was moved cranially along the carotid artery, especially whenever patients smoked. Furthermore, smoking contributed to IMT in the CFA. The effect of homocysteine on arterial wall stiffness was only marginal. Because cross-sectional and prospective studies demonstrate an association between hyperhomocysteinemia and atherosclerotic disease, we suggest that other mechanisms may contribute to vascular obstruction, such as activation of the coagulation cascade or impaired NO generation by the endothelium. It is also possible that homocysteine has an important synergistic effect with other (unknown) factors. A synergistic effect of homocysteine and smoking on arterial wall thickness could not be demonstrated in this study, although some additional decrease in DC was observed when both smoking and hyperhomocysteinemia were present. Furthermore, the effect of homocysteine in genetically inherited disorders may differ from that in the control population.

A criticism on the method used for determining DC and CC in the CCA could be the measurement of pulse pressure at the site of the brachial artery rather than the CCA, which was neglected because of the noninvasive nature of the study. We assumed that the pulse pressure in the brachial artery was representative of CCA pressure. The positive relationship between this pulse pressure and the relative diameter increase of the CCA during systole, as found by Reneman et al, supports this assumption. It is well known that pulse pressure measured on the arm is not representative of pulse pressure in the femoral artery; however, we presumed that the error is systematic and thus does not affect comparative studies.

Because of an inherent disadvantage of a cross-sectional study, the time relationship between structural and functional
vessel wall changes cannot be answered. The obvious correlation between structural and functional changes indicates that they both express the same phenomenon. Long-term follow-up studies in healthy subjects and patients with asymptomatic cardiovascular risk factors are necessary to establish the additional value of measuring functional as well as structural vessel wall properties.

In conclusion, age, LDL cholesterol, blood pressure, and smoking contribute to a variation in arterial wall thickness. The DC of the CCA was influenced by age, LDL cholesterol, and systolic blood pressure. Homocysteine did not explain any variation in IMT and only marginally contributed to the variation in DC in the CCA and in CC in the CFA. Long-term, prospective, follow-up studies in patients homozygous for CBS deficiency and in the general population are necessary to reveal more of the effects of homocysteine on the arterial wall.

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Carotid and Femoral Artery Wall Thickness and Stiffness in Patients at Risk for Cardiovascular Disease, With Special Emphasis on Hyperhomocysteinemia
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