Structure of Cerebral Arterioles in Cystathionine β-Synthase–Deficient Mice

Gary L. Baumbach, Curt D. Sigmund, Teodoro Bottiglieri, Steven R. Lentz

Abstract—We examined effects of hyperhomocysteinemia on structure and mechanics of cerebral arterioles. We measured plasma total homocysteine (tHcy) and pressure, diameter, and cross-sectional area of the vessel wall in maximally dilated cerebral arterioles in heterozygous cystathionine β-synthase–deficient (CBS+/−) mice and wild-type (CBS+/+) littermates that were provided with drinking water that was unsupplemented (control diet) or supplemented with 0.5% L-methionine (high-methionine diet). Plasma tHcy was 5.0±1.1 μmol/L in CBS+/+ mice and 8.3±0.9 μmol/L in CBS+/− mice (P<0.05 versus CBS+/+ mice) fed the control diet. Plasma tHcy was 17.2±4.6 μmol/L in CBS+/+ mice and 21.2±3.9 μmol/L in CBS+/− mice (P<0.05) fed the high-methionine diet. Cross-sectional area of the vessel wall was significantly increased in CBS+/− (437±22 μm²) mice fed control diet and CBS+/+ (442±36 μm²) and CBS+/− (471±46 μm²) mice fed high-methionine diet relative to CBS+/+ (324±18 μm²) mice fed control diet (P<0.05). During maximal dilatation, the stress-strain curves in cerebral arterioles of CBS+/− mice on control diet and CBS+/+ and CBS+/− mice on high-methionine diet were shifted to the right of the curve in cerebral arterioles of CBS+/− mice on control diet, an indication that distensibility of cerebral arterioles was increased in mice with elevated levels of plasma tHcy. Thus, hyperhomocysteinemia in mice was associated with hypertrophy and an increase in distensibility of cerebral arterioles. These findings suggest that hyperhomocysteinemia promotes cerebral vascular hypertrophy and altered cerebral vascular mechanics, both of which may contribute to the increased incidence of stroke associated with hyperhomocysteinemia. (Circ Res. 2002;91:931-937.)

Key Words: hyperhomocysteinemia | cerebral arteriole | vascular hypertrophy

Hyperhomocysteinemia, defined as elevation of plasma total homocysteine (tHcy), is recognized as a prevalent risk factor for a variety of vascular-based diseases.1 Although associations between hyperhomocysteinemia and adverse vascular outcomes have been observed in many retrospective and some prospective studies, the mechanisms responsible for vascular dysfunction in hyperhomocysteinemia remain poorly understood, particularly in the cerebral circulation. Despite strong clinical correlations between hyperhomocysteinemia and carotid intimal thickening and stroke,2 little is known about effects of hyperhomocysteinemia on cerebral vascular structure.

Several lines of evidence suggest that hyperhomocysteinemia may alter vascular structure. First, homocysteine promotes proliferation of rat vascular muscle cells in tissue culture.3 Second, moderate hyperhomocysteinemia is associated with an increased incidence of carotid artery stenosis in humans.4 Third, minipigs fed a methionine-rich casein-based diet for 4 months develop mild hyperhomocysteinemia (∼10 μmol/L) and hyperplasia of aortic muscle.5

Alterations in cerebral vascular structure, and in particular hypertrophy of the vessel wall, are considered major risk factors for stroke. A mechanism by which hypertrophy may contribute to stroke involves encroachment of the vessel wall into the vascular lumen.6 Encroachment displaces vascular muscle into the lumen, thereby resetting the baseline resistance from which vascular muscle exerts its control of vascular resistance to a higher level and impairing responsiveness of cerebral vessels to dilator stimuli.7–9 Thus, by altering cerebral vascular hemodynamics, hypertrophy may increase susceptibility to ischemic stroke during episodes that evoke maximal cerebral vasodilatation. Furthermore, hypertrophy-induced alterations in cerebral hemodynamics may exacerbate the effects of endothelial dysfunction induced by hyperhomocysteinemia.10–12

The goal of this study was to examine effects of hyperhomocysteinemia on structure and mechanics of cerebral arterioles in mice. We hypothesized that cerebral arterioles in hyperhomocysteinemic mice would undergo hypertrophy. In addition, our previous observations13,14 that distensibility of cerebral arterioles is increased in experimental models of chronic hypertension despite arteriolar hypertrophy led us to hypothesize that cerebral arterioles may undergo an increase in distensibility, as well as hypertrophy, during hyperhomocysteinemia.
Materials and Methods

Animals
To minimize the potential influence of differences in genetic background, cystathionine \( \beta \)-synthase (CBS)–deficient mice were crossbred to C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) for at least 7 generations. We studied heterozygous CBS-deficient (CBS\(^{+/−}\)) mice and wild-type (CBS\(^{++}\)) littermates. Homozygous CBS-deficient mice were not studied because they exhibit growth retardation, hepatic dysfunction, and shortened survival. Genotyping for the targeted CBS allele was performed by polymerase-enzyme reaction. At the time of weaning, mice were fed LD-485 chow (Harlan Teklad), which contains 7.5 mg/kg folic acid and 4.0 g/kg L-methionine, and were provided drinking water that was either unsupplemented (control diet) or supplemented with 0.5\% L-methionine (high-methionine diet). The terms “control diet” and “high-methionine diet” refer to the combination of solid chow and drinking water consumed by each group. Mice were studied at 19 to 23 weeks of age. Procedures followed in this study were in accordance with institutional guidelines for care and use of experimental animals at the University of Iowa.

Systemic Arterial Pressure in Conscious Mice
Anesthesia can lower arterial pressure in mice. We therefore measured systemic arterial pressure in conscious mice using a method described previously. For long-term catheterization, mice were anesthetized with Avertin (0.2 to 0.3 mL, IP), shaved, and prepped with a 70% alcohol solution. Sterile catheters (0.040 outer diameter \( \times \) 0.025 inner diameter) were placed in the right carotid artery and the left femoral artery with the aid of a dissecting microscope. Catheters were flushed with a sterile dilute heparin solution (10 U/mL) and were tunneled subcutaneously to the back of the neck. Mice were placed on a warming pad (39°C) during the surgical procedure and postoperatively until fully awake. All animals were given prophylactic antibiotics (penicillin G, 12,000 U IM) and allowed to recover at least 24 hours before any other experimental manipulation. 

In Vivo Preparation
Animals were weighed and anesthetized with sodium pentobarbital (5 mg 100 g\(^{-1}\) body wt IP), intubated, and mechanically ventilated with room air and supplemental O\(_2\). Additional anesthesia (1.7 mg 100 g\(^{-1}\) body wt IV) was administered when pressure to a paw evoked a change in blood pressure or heart rate.

A catheter was inserted into a femoral vein for injection of drugs and fluids. Catheters were inserted into both femoral arteries to record systemic arterial pressure, obtain blood samples for measurement of arterial blood gases, and withdraw blood to produce hypotension (needed for studies of vascular mechanics).

Measurement of Cerebral Arteriolar Pressure and Diameter
We measured pressure and diameter in first order arterioles on the cerebrum through an open skull preparation described previously. A craniotomy was made over the left parietal cortex, and the dura was incised to expose cerebral vessels. Exposed brain was continuously perfused with artificial CSF, warmed to 37°C to 38°C and equilibrated with a gas mixture of 5% CO\(_2\)/95% N\(_2\). Systolic, diastolic, mean, and pulse pressures were measured continuously in cerebral arterioles with a micropipette connected to a servo-null pressure-measuring device (model 5, Instrumentation for Physiology and Medicine Inc). Arterioles were monitored through a microscope connected to a closed-circuit video system with a final magnification of \( \times 356\). Images of arterioles were digitized using a video frame grabber. Arteriolar diameter was measured from the digitized images by the use of image analysis software (NIH Image, National Institutes of Health, Research Services Branch, NIMH). The precision of this system is 0.4 to 0.6 \( \mu \m\), respectively until fully awake. All animals were given prophylactic antibiotics (penicillin G, 12,000 U IM) and allowed to recover at least 24 hours before any other experimental manipulation.

Experimental Protocol
About 20 to 30 minutes after completion of surgery, measurements of cerebral arterioles were obtained under baseline conditions. Vascular muscle was then deactivated by suffusion of cerebral vessels with artificial CSF containing EDTA (67 mmol/L), which produces complete deactivation of smooth muscle in cerebral arterioles. Pressure-diameter relationships were obtained in deactivated cerebral arterioles between cerebral arteriolar pressures of 50 and 10 mm Hg. Hemorrhage was used to reduce cerebral arteriolar pressure in decrements of 10 mm Hg at pressures down to 20 mm Hg and decrements of 5 mm Hg at pressures between 20 and 10 mm Hg. Maximally dilated arterioles were fixed at physiological pressure in vivo by suffusion of vessels with glutaraldehyde fixative (0.0225 glutaraldehyde in 0.10 mol/L cacodylate buffer) while maintaining cerebral arteriolar pressure at baseline levels. After the animal was killed by an injection of potassium chloride, the arterial segment used for pressure-diameter measurements was removed, processed for electron microscopy, and embedded in Spur’s low viscosity resin while cross-sectional orientation was maintained.

Just before killing the animal, blood for measurement of plasma tHcy was removed from a femoral artery. Plasma tHcy, defined as the total concentration of homocysteine after quantitative reductive cleavage of all disulfide bonds, was measured by high performance liquid chromatography and electrochemical detection as described previously.

Calculation of Mechanical Characteristics
The assumptions on which we based calculations of circumferential stress, circumferential strain, and tangential elastic modulus have been described in detail previously. Circumferential stress (\( \sigma \)) was calculated from cerebral arteriolar pressure (\( P \)), inner diameter (\( D_i \)), and wall thickness (WT): \( \sigma = (P \times D_i) / (2 \times WT) \). Cerebral arteriolar pressure was converted from millimeters of mercury to newtons per square meter (1 mm Hg = 1.334 \times 10^2 N/m²). Wall thickness was calculated from cross-sectional area of the vessel wall (CSA) and inner cerebral arteriolar diameter: \( WT = (4 \times CSA / \pi \times D_i^2) / (\pi \times D_i^2) / (2 \times WT) \). External diameter of cerebral arterioles (\( D_e \)) was calculated as \( D_e = D_i \times 2 \times WT \). Circumferential strain (\( \varepsilon \)) was calculated as \( \varepsilon = (D_i - D_o) / D_o \), where \( D_o \) is original diameter (defined as original diameter at 10 mm Hg pressure). To obtain tangential elastic modulus, the stress-strain data from each animal were fitted to an exponential curve (\( y = ae^x \)) using least squares analysis: \( \sigma = \sigma_0 e^{\beta y} \), where \( \sigma_0 \) is stress at original diameter, and \( \beta \) is a constant that is related to the rate of increase of the stress-strain curve.

Determination of Wall Composition
Cross-sectional area of the vessel wall was measured histologically from 1-\( \mu \m\) sections using a light microscope interfaced with the image analyzing system described above. Luminal and total (lumen plus vessel wall) cross-sectional areas of the arteriole were measured by tracing the inner and outer edges of the vessel wall. Cross-sectional area of the arteriolar wall was calculated by subtracting luminal cross-sectional area from total cross-sectional area. Volume density of smooth muscle, elastin, collagen, basement membrane, and endothelium was quantitated from electron micrographs of the vessel wall, using a method that we have described previously. Ultrathin sections of the arteriolar wall were stained with phosphotungstic acid (0.25%) and examined with an electron microscope. Electron micrographs were taken at a magnification of 9000\( \times \) and enlarged by a factor of 3 for a final magnification of 27,000\( \times \). To ensure uniform sampling, the vessel wall was divided into 4 quadrants of equal size. Two or three electron micrographs were taken randomly in each quadrant.

A standard point counting grid was used to count the number of points contained within profiles of smooth muscle, elastin, collagen, basement membrane, and endothelium. Volume density (\( V_v \)) of each component was calculated from the number of points in each component (\( P_v \)) and the total number of points contained within the vessel wall (\( P_t \)): \( V_v = P_v / P_t \). Cross-sectional area of individual wall
components (CSA
\textsubscript{c}) was calculated from \(V_c\) of each component and total cross-sectional area (CSA
\textsubscript{T}): \(CSA
\textsubscript{c}=CSA
\textsubscript{T}×V_c\).

**Statistical Analysis**

Analysis of variance was used to compare systemic mean pressure, arteriolar pressures, diameters, cross-sectional area of the vessel wall, cross-sectional area and volume density of individual components, ratios of nondistensible to distensible components, and slope of tangential elastic modulus versus stress. Probability values were calculated using a Student's t test. Statistics were determined using JMP statistics software (SAS Institute Inc) on a Macintosh computer.

**Results**

**Baseline Values**

Plasma \(\text{tHcy}\) was mildly elevated in CBS\(^{+/-}\) mice fed the control diet (Table 1). In mice fed the high-methionine diet, plasma \(\text{tHcy}\) was moderately elevated in both the CBS\(^{+/-}\) and CBS\(^{+/+}\) groups (Table 1). In the unanesthetized state, systemic arterial mean pressure was not significantly different in CBS\(^{+/-}\) mice and CBS\(^{+/+}\) mice fed the control diet (Table 1). The high-methionine diet did not significantly alter unanesthetized systemic arterial mean pressure in either CBS\(^{+/-}\) or CBS\(^{+/+}\) mice (Table 1). Anesthesia significantly reduced systemic arterial mean pressure in all groups of mice (Table 1). Systemic arterial mean pressure and cerebral arteriolar mean and pulse pressures during anesthesia were not significantly different in any of the groups of mice (Table 1). Diameter before deactivation with EDTA was not significantly different in cerebral arterioles of CBS\(^{+/-}\) or CBS\(^{+/+}\) mice on control diet and was not significantly altered by the high-methionine diet in either group (Table 1). During deactivation with EDTA, internal and external diameters were not significantly different in cerebral arterioles of any of the groups of mice (Table 1). Cross-sectional area of the vessel wall was significantly increased in CBS\(^{+/-}\) mice on control diet, as well as in CBS\(^{+/-}\) and CBS\(^{+/+}\) mice on high-methionine diet, compared with CBS\(^{+/+}\) on control diet (Table 1). Thus, elevated levels of plasma \(\text{tHcy}\) induced by diet and/or CBS-deficiency, either alone or in combination, were associated with hypertrophy of cerebral arterioles.

### TABLE 1. Effect of Diet and CBS Genotype on Baseline Values

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>High-Met Diet</th>
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<tbody>
<tr>
<td></td>
<td>CBS(^{+/-})</td>
<td>CBS(^{+/+})</td>
</tr>
<tr>
<td><strong>Systemic parameters</strong></td>
<td></td>
<td></td>
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<tr>
<td>Plasma total homocysteine, (\mu\text{mol/L})†</td>
<td>5.0±1.1</td>
<td>8.3±0.9*</td>
</tr>
<tr>
<td>Arterial mean pressure, mm Hg</td>
<td>Unanesthetized</td>
<td>113±8</td>
</tr>
<tr>
<td></td>
<td>Anesthetized</td>
<td>74±5</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td>(P_\text{co}_2)</td>
<td>32.2±1.6</td>
</tr>
<tr>
<td></td>
<td>(pH)</td>
<td>7.34±0.02</td>
</tr>
<tr>
<td></td>
<td>(P_\text{o}_2)</td>
<td>146±10</td>
</tr>
<tr>
<td><strong>Cerebral arterioles before EDTA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral arteriolar pressure, mm Hg</td>
<td>Systolic</td>
<td>47±2</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>39±2</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>42±2</td>
</tr>
<tr>
<td></td>
<td>Pulse</td>
<td>8±1</td>
</tr>
<tr>
<td>Internal cerebral arteriolar diameter, (\mu\text{m})</td>
<td>37±3</td>
<td>35±4</td>
</tr>
<tr>
<td><strong>Cerebral arterioles after EDTA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral arteriolar diameter, (\mu\text{m})</td>
<td>Internal</td>
<td>62±5</td>
</tr>
<tr>
<td></td>
<td>External</td>
<td>66±5</td>
</tr>
<tr>
<td>Cross-sectional area of vessel wall, (\mu\text{m}^2)</td>
<td>324±18</td>
<td>437±22*</td>
</tr>
<tr>
<td>(E_t) vs stress</td>
<td>5.44±0.21</td>
<td>4.67±0.22*</td>
</tr>
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Measurements of internal diameter before deactivation of smooth muscle were obtained at prevailing levels of arterial pressure. Measurements of internal diameter after deactivation of smooth muscle were made at an arteriolar mean pressure of 40 \(\mu\text{Hg}\). Values of external diameter after deactivation of smooth muscle were calculated from measurements of internal diameter at 40 \(\mu\text{Hg}\) arteriolar pressure and histological measurements of cross-sectional area of the vessel wall. \(E_t\) vs stress indicates slope of tangential elastic modulus versus stress. Values are mean±SEM. †Plasma total homocysteine was obtained in 10 CBS\(^{+/-}\) and 8 CBS\(^{+/+}\) mice on control diet, and 11 CBS\(^{+/-}\) and 9 CBS\(^{+/+}\) mice on high-methionine diet, whereas all other parameters were obtained in 11 CBS\(^{+/-}\) and 7 CBS\(^{+/+}\) mice on control diet, and 11 CBS\(^{+/-}\) and 9 CBS\(^{+/+}\) mice on high-methionine diet. *\(P<0.05\) vs CBS\(^{+/-}\) mice on control diet.
Figure 1. Pressure-internal diameter (left) and stress-strain relationships (right) in cerebral arterioles during maximal dilatation with EDTA in wild-type (CBS+/+; n=11) and CBS-deficient (CBS−/−; n=7) mice on control diet and CBS−/− (n=13) and CBS−/− (n=10) mice on high-methionine diet. Values are mean±SEM. Closed circles represent CBS+/+ mice on control diet; open circles, CBS+/+ mice on high-methionine diet; closed squares, CBS+/+ mice on control diet; and open squares, CBS+/+ mice on high-methionine diet. D indicates cerebral arteriolar diameter; D0, original cerebral arteriolar diameter.

Vascular Mechanics
The internal diameter of cerebral arterioles during maximal dilatation did not differ significantly between CBS+/+ mice and CBS−/− mice at any level of arteriolar pressure between 50 and 10 μm Hg (Figure 1, left). The high-methionine diet did not significantly alter the degree of maximal dilatation in either CBS+/+ or CBS−/− mice (Figure 1, left). The stress-strain curves in cerebral arterioles in CBS+/+ mice on control diet and CBS+/+ and CBS+/+ mice on high-methionine diet were shifted to the right of the curve in CBS−/− mice on control diet (Figure 1, right). The slope of tangential elastic modulus versus stress was significantly decreased in CBS+/+ mice on control diet, as well as in CBS+/+ and CBS+/+ mice on high-methionine diet. These findings suggest that hyperhomocysteinemia in mice was accompanied by increases in passive distensibility of cerebral arterioles.

Vascular Composition
The composition of cerebral arterioles was qualitatively similar in CBS+/+ mice on control diet and CBS−/− mice on high-methionine diet (Figure 2), and consisted of 5 major components: endothelium, smooth muscle, elastin, basement membrane, and collagen. Most of the hypertrophy that occurred in cerebral arterioles of CBS+/+ mice on high-methionine diet resulted from increases in cross-sectional area of smooth muscle and elastin, whereas cross-sectional area of collagen, basement membrane, and endothelium did not increase significantly (Table 2). Whereas volume densities of smooth muscle, collagen, and basement membrane were not altered in cerebral arterioles of CBS+/+ mice fed the high-methionine diet, volume density of elastin increased by a factor of two (Table 2). Thus, hypertrophy of cerebral arterioles in hyperhomocysteinemic mice resulted primarily from increases in the more distensible components of the arteriolar wall, smooth muscle and elastin, whereas the stiffer components, collagen and basement membrane, contributed little to the increase in wall mass.

Table 2. Effect of Diet and Genotype on Composition of Cerebral Arterioles

<table>
<thead>
<tr>
<th>Component</th>
<th>Control Diet CBS+/+</th>
<th>High-Met Diet CBS−/−</th>
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<tr>
<td>Cross-sectional area, μm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>149±16</td>
<td>221±19*</td>
</tr>
<tr>
<td>Elastin</td>
<td>28±3</td>
<td>89±9*</td>
</tr>
<tr>
<td>Collagen</td>
<td>2±0.6</td>
<td>2±0.3</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>28±2</td>
<td>37±4</td>
</tr>
<tr>
<td>Endothelium</td>
<td>117±10</td>
<td>122±18</td>
</tr>
<tr>
<td>Volume density, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>46±3</td>
<td>47±2</td>
</tr>
<tr>
<td>Elastin</td>
<td>9±1</td>
<td>19±2*</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.3±0.05</td>
<td>0.4±0.07</td>
</tr>
<tr>
<td>Basement membrane</td>
<td>9±1</td>
<td>8±1</td>
</tr>
<tr>
<td>Endothelium</td>
<td>36±4</td>
<td>26±3*</td>
</tr>
</tbody>
</table>

Components of arterioles that were used for in vivo determination of vascular mechanics.
Values are mean±SEM in 11 CBS+/+ mice on control diet and 9 CBS+/+ mice on 0.5% high methionine diet. *P<0.05 vs CBS+/+ mice.
To relate alterations in structure of cerebral arterioles to alterations in distensibility, we calculated the ratio of nondistensible to distensible components in the arteriolar wall. Basement membrane was added to collagen, rather than to elastin, because basement membrane contains significant amounts of type IV collagen.22 Smooth muscle was combined with elastin because they have similar elastic moduli.23 The ratio of basement membrane and collagen to elastin in cerebral arterioles again was lower in CBS+/− mice fed high-methionine diet than in CBS+/+ mice fed control diet (Figure 3, left). The ratio of basement membrane and collagen to smooth muscle and elastin was also lower in CBS+/− mice fed high-methionine diet (Figure 3, right). Thus, hypertrophy of cerebral arterioles with hyperhomocysteinemia was accompanied by a relative increase in the more distensible components of the arteriolar wall.

Discussion

There are three major new findings in this study. First, mild-to-moderate hyperhomocysteinemia produced in mice by high-methionine diet, heterozygous CBS-deficiency, or a combination of the two, was associated with hypertrophy of cerebral arterioles. To our knowledge, this study is the first to demonstrate that hyperhomocysteinemia may result in cerebral vascular hypertrophy (and possibly hypertrophy in any vascular bed). Second, hyperhomocysteinemia was associated with increased cerebral arteriolar distensibility. Third, increases in distensibility during hyperhomocysteinemia were associated with increases in the more distensible components in the vessel wall.

Alterations in Vascular Structure

The possibility that experimental induction of hyperhomocysteinemia may alter vascular structure was suggested by observations in minipigs that were fed a methionine-rich diet.5 Hyperhomocysteinemia in minipigs was associated with evidence of smooth muscle hyperplasia in the tunica media of aorta, carotid artery, coronary artery, and intramuscular small resistance arteries.5 Our observations in this study indicate that experimental hyperhomocysteinemia also produces vascular hypertrophy in mice.

The combinations of high-methionine and control diets in wild-type and CBS-deficient mice resulted in graded increases in plasma tHcy in the three groups of hyperhomocysteinemic mice (increases in plasma tHcy relative to CBS+/+ mice on control diet were about 3 μmol/L in CBS+/− mice on control diet, 12 μmol/L in CBS+/− mice on high-methionine diet, and 16 μmol/L in CBS+/− on high-methionine diet). On the other hand, cross-sectional area of vessel wall was not increased by similar amounts in the three groups of hyperhomocysteinemic mice (see Table 1). These findings suggest a threshold effect of hyperhomocysteinemia on cerebral vascular structure in mice rather than the graded effect that we had anticipated.

We considered 3 possible mechanisms that may have contributed to the development of cerebral vascular hypertrophy. First, hyperhomocysteinemia may promote cerebral vascular hypertrophy directly by stimulating growth of smooth muscle in the arteriolar wall. This possibility is supported by the observation that homocysteine promotes growth of vascular muscle cells in tissue culture in a dose-dependent manner.3 Second, hyperhomocysteinemia may induce cerebral vascular hypertrophy indirectly through endothelial injury. We and others have shown previously that endothelium-dependent, but not endothelium-independent, dilatation is impaired in aorta10,11 and mesenteric vessels of hyperhomocysteinemic mice,12 and middle cerebral artery in humans with methionine-induced hyperhomocysteinemia.24 These findings combined with the findings that nitric oxide suppresses mitogenesis and proliferation of vascular muscle cells in tissue culture25 and inhibition of nitric oxide synthase promotes hypertrophy of cerebral arterioles26 suggests that hyperhomocysteinemia may promote growth of cerebral vascular muscle indirectly by reducing availability of nitric oxide and thus diminishing its growth inhibitory influence. The third possibility we considered is that hyperhomocysteinemia may promote cerebral vascular hypertrophy indirectly through increases in arterial pressure, in particular pulse pressure. The rationale for this possibility is based on the finding in this study that hyperhomocysteinemia was sufficient to produce hypertrophy of cerebral arterioles in rats, even in the absence of increases in cerebral arteriolar mean pressure.27 This possibility is unlikely, however, based on the finding in this study that hyperhomocysteinemia was not associated with increases in cerebral arteriolar pulse pressure in anesthetized mice.

The possible consequences of cerebral vascular hypertrophy are 2-fold. First, hypertrophy of cerebral blood vessels during hyperhomocysteinemia may shift cerebral vascular autoregulation to a higher range of arterial pressure. This supposition is based on the finding that cerebral vascular hypertrophy during chronic hypertension is associated with a rightward shift in cerebral vascular autoregulation.28 The mechanism by which hypertrophy of the vessel wall contributes to alterations in autoregulation is thought to be related to encroachment of the hypertrophied vessel wall into the vascular lumen, which in turn resets cerebral vascular resistance to a higher level.6 The shift in cerebral autoregulation

![Figure 3. Ratio of collagen plus basement membrane to elastin ([C+BSM]:Elastin) and collagen plus basement membrane to elastin plus smooth muscle ([C+BSM]:[E+SM]) in cerebral arterioles of CBS+/+ mice fed control diet and CBS+/− mice fed high-methionine diet. *P<0.05 vs CBS+/+ on control diet.](http://circres.ahajournals.org/)

![Image](http://circres.ahajournals.org/)
impairs autoregulatory vasodilatation in response to acute hypotension. Thus, impairment of autoregulatory vasodilatation by cerebral vascular hypertrophy may be another mechanism, in addition to impaired endothelium-dependent cerebral vasodilatation, by which hyperhomocysteinemia contributes to an increased incidence of stroke.

Another consequence of cerebral vascular hypertrophy relates to its effect on wall stress. By virtue of an increase in wall thickness, vascular hypertrophy reduces wall stress.29 Cerebral vascular hypertrophy during chronic hypertension normalizes increases in wall stress that would otherwise occur without hypertrophy of the vessel wall due to chronically elevated arterial pressure. Although arterial pressure may not increase in response to hyperhomocysteinemia, hyperhomocysteinemia may alter vascular structure in ways that render the vessel wall more susceptible to stress-related damage, in which case reducing wall stress through hypertrophy may be protective.

**Distensibility and Wall Composition**

Several investigators have examined the relationship between hyperhomocysteinemia and vascular distensibility. Bortolotto et al30 found that elevations of plasma homocysteine levels in hypertensive patients correlate strongly with reductions in aortic distensibility independently of age, blood pressure, extent of atherosclerosis, and renal function. Lambert et al11 and Van Guldener et al,32,33 on the other hand, found no relationship between levels of plasma tHcy and distensibility of brachial arteries in first-degree relatives of patients with familial hyperhomocysteinemia or in patients on chronic hemodialysis. Furthermore, Smilde et al34 observed that in patients with known cardiovascular risk factors (familial hypercholesterolemia, hypertension, or smoking), increases in plasma tHcy accounted for only a small proportion of the reductions in distensibility in carotid and femoral arteries with the major proportion due to age and smoking. In contrast to studies in humans, Rolland et al5 found that hyperhomocysteinemia in minipigs resulted in increased distensibility in cerebral arterioles and in other species, such as minipigs and mice.

The finding in this study that hypertrophy of cerebral arterioles is accompanied by an increase in distensibility may seem paradoxical. Intuition suggests that the increase in thickness of the vessel wall that results from hypertrophy would lead to a reduction rather than an increase in vascular distensibility. Despite the apparent paradox, however, there are several other examples of an association between vascular hypertrophy and increased distensibility. We found previously that cerebral arterioles in several experimental models of chronic hypertension, including spontaneously hypertensive rats (SHR), stroke-prone SHR, and rats with 1-kidney, 1-clip renal hypertension, undergo both hypertrophy and increased distensibility.14,18 In addition, hypertrophy of radial artery in humans with essential hypertension and carotid artery in rats with 2-kidney, 1-clip hypertension is accompanied by an increase in distensibility in both cases.35,36

We have proposed previously that increases in passive distensibility that accompany hypertrophy of cerebral arterioles may be due to alterations in proportional composition of the arteriolar wall.13,14 The finding in this study that hypertrophy of cerebral arterioles in hyperhomocysteinemic mice is accompanied by a relative increase in the more compliant components of the arteriolar wall provides additional support for this concept. The association of alterations in composition and mechanics of cerebral vessels, however, may be coincidental and therefore must be interpreted with caution. In some vessels, alterations in composition may not be predictive of alterations in vascular mechanics. For example, distensibility of the internal carotid artery is decreased in SHR, despite a reduction in the ratio of collagen to elastin.37

Another consideration is that effects of hyperhomocysteinemia on composition of the vessel wall may be different in small resistance arteries and arterioles than in large arteries. Vermeulen et al38 found a reduction in the vascular smooth muscle cell/extracellular matrix (VSM/ECM) ratio in femoral arteries of patients with hyperhomocysteinemia. In the present study, the ratio of VSM/ECM (determined as sum of collagen and basement membrane from Table 2) in cerebral arterioles was 5.0±0.4 in tunica media of cerebral arterioles in CBS-/- on control diet mice compared with 5.7±0.4 in CBS-/- on high-methionine diet. Thus, hyperhomocysteinemia in mice tended to increase the VSM/ECM ratio in cerebral arterioles. Of course, other explanations are possible. For example, substantially different methods were used in the two studies, different vascular beds were examined (peripheral vasculature versus cerebral), and the patients in the Vermeulen study38 had atherosclerosis involving femoral artery, whereas the mice in this study were not atherosclerotic. Nevertheless, differences in vessel size may be important with respect to effects of hyperhomocysteinemia on vessel wall composition.

In conclusion, this study demonstrates that hyperhomocysteinemia in mice leads to hypertrophy of cerebral arterioles accompanied by a reduction in the ratio of nondistensible-to-distensible components and an increase in distensibility. We speculate that hypertrophy of cerebral arterioles may play a role, along with impaired endothelium-dependent dilatation,
in the increased susceptibility to stroke that is associated with hyperhomocysteinemia.

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Structure of Cerebral Arterioles in Cystathionine β-Synthase-Deficient Mice
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