Age-Related Effects on Atherogenesis and Scavenger Enzymes of Intracranial and Extracranial Arteries in Men Without Classic Risk Factors for Atherosclerosis

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Background and Purpose—Atherosclerosis occurs later and is less extensive in intracranial arteries than in extracranial arteries. However, the mechanisms responsible are poorly understood. A previous study has suggested a better antioxidant protection of intracranial arteries.

Methods—To assess the influence of age on arterial activity of antioxidant enzymes and atherogenesis, we compared intracranial and extracranial arteries of humans of different ages who retrospectively lacked confounding classic risk factors (48 premature fetuses aged 6.4±0.8 months [mean±SD], 58 children aged 7.9±3.8 years, 42 adults aged 42.5±5.1 years, and 40 elderly subjects aged 71.8±3.4 years; all males). Lesions were quantified by computer-assisted imaging analysis of sections of the middle cerebral and basilar arteries, the left anterior descending coronary artery, the common carotid artery, and the abdominal aorta. Macrophages, apolipoprotein B, oxidized LDL, and matrix metalloproteinase-9 in lesions were determined by immunocytochemistry. The effect of aging on atherogenesis was then compared with that on the activity of 4 antioxidant enzymes in the arterial wall.

Results—Atherosclerosis was 6- to 19-fold greater (P<0.01) in extracranial arteries than in intracranial arteries, and it increased linearly with age. Intracranial arteries showed significantly greater antioxidant enzyme activities than did extracranial arteries. However, the antioxidant protection of intracranial arteries decreased significantly in older age, coinciding with a marked acceleration of atherogenesis. An increase in matrix metalloproteinase-9 protein expression and in gelatinolytic activity consistent with the degree of intracranial atherosclerosis was also observed.

Conclusions—These results suggest that a greater activity of antioxidant enzymes in intracranial arteries may contribute to their greater resistance to atherogenesis and that with increasing age intracranial arteries respond with accelerated atherogenesis when their antioxidant protection decreases relatively more than that of extracranial arteries. (Stroke. 2001;32:2472-2480.)

Key Words: atherosclerosis ■ cerebral arteries ■ lipoproteins, LDL ■ oxygen radical

Despite progress in prevention, ischemic stroke remains the third most common cause of death in the Western world, after coronary heart disease and cancer.1 The role of hypercholesterolemia in atherosclerotic cerebrovascular disease is still unclear.2,3 Statins reduce the incidence of nonfatal stroke in coronary heart disease patients1–3 but are apparently less effective in reducing the mortality from ischemic stroke.1–3 Although intracranial arteries eventually develop atherosclerotic lesions, the onset of atherogenesis occurs much later in life, and the severity of the lesions at various ages is consistently less than that in extracranial arteries in humans,2,4–9 nonhuman primates,10 rhesus and cynomolgus monkeys,11 spontaneously hypertensive rats,12,13 cholesterol-fed rabbits,11 and Watanabe heritable hyperlipidemic rabbits.14 To date, it is unknown whether the difference in atherosclerosis is due to anatomic differences between intracranial and extracranial arteries, systemic differences (eg, lower local blood pressure), or other differences in atherogenic mechanisms. LDL oxidation is thought to affect many atherogenic mechanisms.15 In a preceding study,9 we reported that the activity of antioxidant enzymes, in particular the oxygen-radical scavenger manganese superoxide dismutase (Mn-SOD), tended to be consistently greater in intracranial arteries of premature human fetuses than in extracranial arteries. However, it is not known whether this...
difference persists after birth. One of the mechanisms by which enhanced lipid peroxidation could affect atherogenesis and plaque rupture would be by enhancing the expression of matrix metalloproteinases (MMPs). Oxygen radicals and oxidized LDLs (oxLDLs) have both been shown to enhance MMP-9 activity, and human carotid unstable plaques undergoing spontaneous embolization have increased MMP-9 activity. To investigate whether differences in arterial antioxidant protection may contribute to the different susceptibility to atherogenesis and to assess the impact of aging on both parameters, we compared the effect of age on human intracranial and extracranial arteries in subjects lacking classic risk factors of atherosclerosis as far as retrospective data could prove.

Subjects and Methods

Human Subjects

Arteries were obtained at autopsy from spontaneously aborted fetuses (fetal age 6.4±0.8 months, n=48,20,21 including 42 fetuses that were part of our previous studies,20,21 58 children (aged 7.9±3.8 years), including 21 that were part of the Fate of Early Lesions in Children (FELIC) study,21 42 adults (aged 42.5±5.1 years), and 40 elderly subjects (aged 71.8±3.4 years) who underwent routine autopsy at Federico II University of Naples. The study protocol was approved by the local human ethics committee.

We previously reported that maternal hypercholesterolemia during pregnancy was associated with greatly increased lesions in the aorta of the fetus and faster progression of atherosclerosis in their normocholesterolemic children.21 Because the relative frequency of maternal hypercholesterolemia in adults and elderly subjects has not been established, we arbitrarily included an equal number fetuses from normocholesterolemic and hypercholesterolemic mothers (n=24 each). Similarly, we included equal numbers of male children of both groups of mothers (n=29 each). Note that the children themselves were normocholesterolemic. Causes of death in adult and elderly men were trauma (n=57) or liver cirrhosis (n=29 each). Note that the children from normocholesterolemic and hypercholesterolemic mothers were obtained retrospectively, detailed medical histories, clinical records, and blood samples obtained from all subjects shortly before death or at the time of autopsy permitted us to establish with some degree of confidence that none of the subjects had the classic risk factors for atherosclerosis (family history for coronary heart disease, diabetes, smoking, hypertension, and hyperlipidemia) or manifest atherosclerosis-related diseases. Plasma vitamin E was measured by high-performance liquid chromatography, as previously described.22

Preparation of Arterial Sections, Histological and Immunohistochemical Analyses, and Zymography

Representative segments of the abdominal aorta and the entire common carotid, left anterior descending coronary (LAD), basilar, and middle cerebral arteries were dissected, cut open, washed thoroughly with cold sterile PBS, and placed in ice-cold PBS containing 50 μmol/L butylated hydroxytoluene, 0.001% aprotinin, 50 mmol/L EDTA, and 0.008% chloramphenicol, equilibrated with nitrogen, as described.20,21 For each subject, one segment of each artery was immersed in optimal cutting temperature (OCT) compound and flash-frozen in liquid nitrogen, and 30 to 40 sections per segment (7 μm thick) were prepared for morphometry of the lesions.20,21 Cryosections were stained with oil red O and counterstained with hematoxylin. The cumulative area of all lesions (oil red O-positive areas) per section was then determined by computer-assisted image analysis. To permit a direct comparison of lesion formation between arteries of different size, data were then corrected by dividing the cumulative lesion area by the average outer circumference of each artery. Another segment from the same artery of each patient was fixed in buffered 10% formalin and paraffin-embedded, and 12 to 15 serial sections (5 to 7 μm thick) were prepared for immunocytochemistry, as described.20,21 Serial sections were stained with the following: (1) MDA2, a murine monoclonal antibody against malondialdehyde (MDA)-lysozepoxide of oxLDL; (2) NP1539, a mouse monoclonal antibody (IgG1) to human apolipoprotein B (Boehringer-Mannheim Italia); and (3) HAM-56, a monoclonal antibody against human macrophage/foam cells (Accell Accurate). MMP-9 was detected with a mouse monoclonal antibody against the active form of MMP-9 (Oncogene Science) that was also used for Western blot analysis in arterial whole-cell extracts, as previously described.22 All antibodies were used at a dilution of 1:500. Epitopes recognized by the primary antibody were detected by an avidin-biotin-peroxidase method.20,21 MMP zymography (measuring gelatinolytic activity) was performed after homogenization of 4 to 9 μg arterial tissue, as described,24 and results were normalized to total protein by Bradford assay (Bio-Rad Laboratories). Briefly, the composition of enzyme assay buffer for the development of enzyme activity bands was as follows: Tris (3.02 g/L), CaCl2 (0.75 g/L), NaCl (0.9 g/L), and Na2N (0.5 g/L), at pH 7.5. After incubation, the gels were stained with Coomassie brilliant blue, and gelatinolytic activities were detected as transparent bands against the background of Coomassie-stained gelatin. The intensity of the zymogram bands was expressed as arbitrary units and analyzed by densitometry.23

Determination of Antioxidant Enzymes in the Arterial Wall

Additional arterial segments were homogenized in potassium phosphate buffer, pH 7.4, containing 10 μmol/L deferoxamine, 0.03% butylated hydroxytoluene, and 2% ethanol, equilibrated with nitrogen (to reduce autoxidation), and centrifuged at 1000g for 15 minutes at 4°C to remove nuclei and tissue debris. The supernatant was centrifuged again at 3000g for 35 minutes at 4°C. Glutathione peroxidase, catalase, copper-zinc superoxide dismutase (CuZn-SOD), and Mn-SOD tissue activities, normalized for the protein content, were determined spectrophotometrically, as described.

Statistical Analysis

Results were analyzed by 1-way ANOVA followed by Bonferroni correction. A value of P<0.05 was considered significant. Numerical data obtained from immunohistochemistry were analyzed for mean, variance, standard deviation, kurtosis, and skew. To control for the differences in arterial size, we used age-matched arterial segments.
for the effect of age and plasma cholesterol, glucose, and vitamin E concentration on cumulative lesion areas, multiple regression analyses was performed, and $\beta$ coefficients are presented. Correlations between the results were also evaluated by linear regression analysis. All data were analyzed by SPSS statistical software (SPSS Inc).

### Results

#### Extent of Atherosclerosis

Morphometric evaluation of atherosclerosis in extracranial and intracranial arteries revealed that lesions in each artery increased with increasing age. Figure 1 shows results expressed as corrected cumulative lesion area per section, a parameter that allows comparison of arteries of different caliber. The noncorrected cumulative lesion sizes are indicated in boldface.

### TABLE 1. Multiple Regression Analysis Evaluating the Effect of Age, Plasma Cholesterol, Plasma Glucose, and Plasma Vitamin E on Lesion Sizes in Intracranial and Extracranial Arteries of Adults and Elderly Subjects

<table>
<thead>
<tr>
<th></th>
<th>MCA</th>
<th>Basilar</th>
<th>LAD</th>
<th>Carotid</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>$P$</td>
<td>$\beta$</td>
<td>$P$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>No liver cirrhosis Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2.199</td>
<td>0.033</td>
<td>6.329</td>
<td>0.021</td>
<td>1.155</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.898</td>
<td>0.456</td>
<td>5.111</td>
<td>0.723</td>
<td>4.106</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.271</td>
<td>0.656</td>
<td>5.533</td>
<td>0.654</td>
<td>5.111</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>-2.222</td>
<td>0.013</td>
<td>-6.938</td>
<td>0.008</td>
<td>-9.849</td>
</tr>
<tr>
<td>Elderly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.169</td>
<td>0.020</td>
<td>1.192</td>
<td>0.009</td>
<td>1.900</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.523</td>
<td>0.323</td>
<td>1.099</td>
<td>0.412</td>
<td>1.877</td>
</tr>
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<td>Cholesterol</td>
<td>2.918</td>
<td>0.258</td>
<td>1.757</td>
<td>0.342</td>
<td>2.678</td>
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<tr>
<td>Vitamin E</td>
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<td>0.006</td>
<td>-5.786</td>
<td>0.001</td>
<td>-5.495</td>
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<tr>
<td>Liver cirrhosis Adults (n=16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2.044</td>
<td>0.038</td>
<td>4.785</td>
<td>0.036</td>
<td>2.342</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.123</td>
<td>0.321</td>
<td>5.865</td>
<td>0.675</td>
<td>3.008</td>
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<tr>
<td>Cholesterol</td>
<td>4.004</td>
<td>0.512</td>
<td>4.876</td>
<td>0.554</td>
<td>3.777</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>-2.003</td>
<td>0.024</td>
<td>-6.003</td>
<td>0.012</td>
<td>-6.821</td>
</tr>
<tr>
<td>Elderly (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.204</td>
<td>0.013</td>
<td>1.876</td>
<td>0.010</td>
<td>2.006</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.342</td>
<td>0.128</td>
<td>2.004</td>
<td>0.275</td>
<td>2.223</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.453</td>
<td>0.321</td>
<td>1.998</td>
<td>0.288</td>
<td>3.234</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>-3.004</td>
<td>0.018</td>
<td>-4.112</td>
<td>0.021</td>
<td>-5.072</td>
</tr>
</tbody>
</table>

MCA indicates middle cerebral artery; AA, abdominal aorta. Data for subjects without and with cirrhosis are presented separately, because an influence of coagulopathies in cirrhotic patients on atherogenesis could not be ruled out a priori. Significant $P$ values are indicated in boldface.

As expected from the exclusion of dyslipidemic and diabetic subjects, multiple regression analysis showed that plasma cholesterol and glucose of the adult and elderly groups did not influence atherogenesis (Table 1). However, plasma cholesterol levels were an independent risk factor for LAD lesions. More important, age was independently related to atherogenesis in both intracranial and extracranial arteries in the adult group and in elderly men, except for the aorta (Table 1). Plasma vitamin E concentrations were inversely correlated with atherogenesis of brain arteries in adult and elderly groups; this effect was also seen in LAD and carotid arteries but not in the aorta (Table 1). A separate analysis of adult

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Liver cirrhosis
(n=16) and elderly men (n=9) who died of liver cirrhosis showed similar results (Table 1).

**Immunohistochemistry**

Paraffin-embedded serial sections of arteries from the study population were immunostained and assessed for the intimal presence of apolipoprotein B, oxLDL, macrophage-derived foam cells, and MMP-9. Results are shown in Figure 2. The abdominal aorta, common carotid artery, and LAD showed significantly more staining for LDL, oxLDL, foam cells, and MMP-9 than did the middle cerebral and basilar arteries. This was in agreement with the smaller numbers and sizes of lesions in intracranial arteries compared with extracranial arteries. In each artery, there was an age-related increase of staining for all epitopes. Only in the elderly group did brain arteries show a marked staining for oxLDL, MMP-9, and foam cells. Staining of the same section with differently labeled detection antibodies indicated colocalization between oxLDL and MMP-9 in basilar and middle cerebral arteries of adults (r=0.41 and 0.35, respectively; P<0.04) and elderly men (r=0.48 and 0.41, respectively; P<0.01). Both endothelial and smooth muscle cells (Figure 3, top panel, arrowheads) showed immunostaining for MMP-9.

**MMP-9 Activity**

Evidence of an age-related increase of MMP-9 activity in brain arteries was also provided by Western blot analysis and by zymography in whole-cell extracts, which showed an increase in MMP-9 protein expression and gelatinolytic activity consistent with the degree (class I, II, or III lesions) of atherosclerotic lesions (Figure 3, bottom panel).

**Tissue Scavenger Enzymes**

To investigate whether differences in lipid peroxidation may contribute to the greater resistance of intracranial arteries to atherogenesis, we determined the arterial activities of glutathione peroxidase, catalase, CuZn-SOD, and Mn-SOD. As evident in Table 2, both intracranial arteries showed much better antioxidant protection than did extracranial arteries until the adult age. In contrast, the content of all antioxidants in intracranial arteries significantly decreased in the elderly group. Glutathione peroxidase was inversely correlated with atherosclerotic lesion size in the middle cerebral artery (r=-0.56, P<0.002) and basilar artery (r=-0.61, P<0.001) in the elderly group. Mn-SOD activity was also correlated with lesion sizes in the middle cerebral artery (r=-0.71, P<0.0008) and the basilar artery (r=-0.77, P<0.0005).
To further examine the potential connection between antioxidant defenses in the arterial wall and lesion sizes, the vascular activity of Mn-SOD was plotted over age (Figure 4). Although in each age group data appeared to be clustered, linear regression analysis indicated that there was a strong inverse correlation between Mn-SOD activity in the 2 intracranial arteries but not in the extracranial arteries (data for the LAD are not shown but closely resembled data for the carotid

Table 2. Comparison of the Total Activity of Scavenger Enzymes in Homogenates of Intracranial and Extracranial Arteries of Humans of Different Ages

<table>
<thead>
<tr>
<th></th>
<th>MCA</th>
<th>Basilar Artery</th>
<th>LAD</th>
<th>Carotid Artery</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetuses Glutathione peroxidase, mU/mg protein</td>
<td>84±16</td>
<td>80±11</td>
<td>72±12</td>
<td>68±14</td>
<td>70±11</td>
</tr>
<tr>
<td>Catalase, IU/mg protein</td>
<td>19±6</td>
<td>18±5</td>
<td>15±6</td>
<td>14±7</td>
<td>18±5</td>
</tr>
<tr>
<td>CuZn-SOD, IU/mg protein</td>
<td>6.5±0.9</td>
<td>6.6±0.9</td>
<td>6.2±0.8</td>
<td>7.2±1.0</td>
<td>6.8±0.9</td>
</tr>
<tr>
<td>Mn-SOD, IU/mg protein</td>
<td>3.1±0.6*</td>
<td>3.2±0.6*</td>
<td>1.5±0.4</td>
<td>1.6±0.4</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>Children Glutathione peroxidase, mU/mg protein</td>
<td>90±12</td>
<td>91±14</td>
<td>85±15</td>
<td>80±16</td>
<td>81±15</td>
</tr>
<tr>
<td>Catalase, IU/mg protein</td>
<td>19±8</td>
<td>20±6</td>
<td>19±6</td>
<td>18±7</td>
<td>19±7</td>
</tr>
<tr>
<td>CuZn-SOD, IU/mg protein</td>
<td>7.5±1.1</td>
<td>7.0±1.1</td>
<td>6.5±0.9</td>
<td>7.5±1.4</td>
<td>7.0±1.2</td>
</tr>
<tr>
<td>Mn-SOD, IU/mg protein</td>
<td>3.4±0.8*</td>
<td>3.6±0.7*</td>
<td>1.6±0.5</td>
<td>1.8±0.5</td>
<td>1.9±0.5</td>
</tr>
<tr>
<td>Adults Glutathione peroxidase, mU/mg protein</td>
<td>78±12</td>
<td>75±13</td>
<td>65±12‡</td>
<td>61±10‡</td>
<td>68±12</td>
</tr>
<tr>
<td>Catalase, IU/mg protein</td>
<td>14±3</td>
<td>15±4</td>
<td>12±4‡</td>
<td>11±5‡</td>
<td>13±4</td>
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<tr>
<td>CuZn-SOD, IU/mg protein</td>
<td>5.9±0.6</td>
<td>5.6±0.8</td>
<td>5.2±0.4‡</td>
<td>5.0±0.8‡</td>
<td>5.2±0.5</td>
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<tr>
<td>Mn-SOD, IU/mg protein</td>
<td>2.4±0.4*</td>
<td>2.3±0.4*</td>
<td>1.4±0.3</td>
<td>1.5±0.4</td>
<td>1.7±0.4</td>
</tr>
<tr>
<td>Elderly Glutathione peroxidase, mU/mg protein</td>
<td>62±11†</td>
<td>60±10†</td>
<td>48±11†</td>
<td>57±12‡</td>
<td>58±11‡</td>
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<td>Catalase, IU/mg protein</td>
<td>13±5†</td>
<td>14±3†</td>
<td>11±3†</td>
<td>12±5‡</td>
<td>14±4</td>
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<tr>
<td>CuZn-SOD, IU/mg protein</td>
<td>5.4±0.5†</td>
<td>5.6±0.8</td>
<td>4.8±0.6‡</td>
<td>4.6±0.8‡</td>
<td>4.8±0.7</td>
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<tr>
<td>Mn-SOD, IU/mg protein</td>
<td>1.8±0.4†</td>
<td>1.6±0.3†</td>
<td>1.0±0.3†</td>
<td>1.3±0.5</td>
<td>1.5±0.4</td>
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</tbody>
</table>

Values are mean±SD. 
*P<0.05 vs LAD, carotid artery, and AA; †P<0.05 vs fetuses and children; and ‡P<0.05 vs children.

To further examine the potential connection between antioxidant defenses in the arterial wall and lesion sizes, the vascular activity of Mn-SOD was plotted over age (Figure 4). Although in each age group data appeared to be clustered, linear regression analysis indicated that there was a strong inverse correlation between Mn-SOD activity in the 2 intracranial arteries but not in the extracranial arteries (data for the LAD are not shown but closely resembled data for the carotid...
artery and abdominal aorta). Indeed, only a minor influence of aging was evident in the extracranial arteries ($r^2 = 0.16$ and $0.37$, respectively) compared with the intracranial arteries ($r^2 = 0.71$ and $0.68$).

**Lesion Progression Over Time**

When the size of atherosclerotic lesions of all subjects was similarly plotted over age (Figure 5), data of the carotid artery and abdominal aorta were best fitted by a linear regression line. This is consistent with the linear progression of overall aortic lesions during childhood and infancy reported by the FELIC study. In contrast, in both intracranial arteries, data followed an exponential curve, with the most apparent acceleration occurring in the elderly subjects.

**Discussion**

The present study represents the first systematic comparison of intracranial and extracranial atherogenesis in humans ranging from premature fetuses to elderly subjects. None of the study subjects had confounding classic risk factors of the disease, as far as could be ascertained retrospectively. The main results are (1) that intracranial arteries generally contain higher activities of antioxidant (oxygen radical scavenger) enzymes than do extracranial arteries, (2) that the antioxidant protection of intracranial arteries markedly decreases with increasing age, and (3) that this coincides with a rapid acceleration of atherosclerosis in intracranial arteries of elderly subjects, whereas the progression of atherogenesis in extracranial arteries is linear over all ages. This suggests that the progression of atherosclerosis in intracranial arteries of older men may in part be due to reduced intracellular defenses against oxygen radical–mediated processes. Increased levels of in situ MMP-9 activity are consistent with this assumption, because oxLDL is known to promote MMP-9 expression. These findings are noteworthy, because they indicate a potential role of lipid peroxidation even in subjects without conventional risk factors, in particular without hypercholesterolemia or dyslipidemia.

Our findings are consistent with several previous observations. For example, the presence of oxLDL in lesions significantly increases with advancing age, and plasma LDL also becomes more susceptible to oxidation. This may be a consequence of the age-related reduced capability of intracellular defenses against oxygen radical–mediated processes. As we now show, glutathione peroxidase (one of the most important antioxidative enzymes in the brain), Mn-SOD, CuZn-SOD, and catalase, were significantly decreased in intracranial arteries of elderly men. Clearly, the relative contributions of these potential mechanisms to atherogenesis must be addressed in experimental models of the disease rather than in postmortem tissues. However, in vitro exposure to oxLDL has been shown to result in impaired vasodilatation of carotid arteries but not basilar arteries, suggesting that the differences in arterial enzyme activities may be functionally relevant. Recent data in stroke-prone hypertensive rats have demonstrated that exogenous administration of the antioxidant, vitamin E, or calcium antagonists with antioxidant properties reduces their long-term mortality. On the other hand, studies in humans have yielded conflicting effects of vitamin E treatment on clinical end points (see review). However, atherogenesis is a complex disease, and it is possible that the lower susceptibility of intracranial arteries to cholesterol-induced atherogenesis results mainly from the coincidence of lower blood pressure and decreased susceptibility to endothelial dysfunction. Reduced blood-brain barrier permeability to LDL and oxLDL may also account for the lesser atherogenic response of intracranial arteries to hypercholesterolemia.

The present study also demonstrates that MMP-9 expression in brain arteries progressively increases with age. MMP-9 activity is correlated with human carotid plaque
instability.19 Because oxLDL upregulates MMP-9 expression18 and because we found a colocalization of MMP-9 and oxLDL in atherosclerotic lesions and age-related increases in both oxLDL and MMP-9 gelatinolytic activity, it is possible that age-related MMP-9 overexpression may play a role in plaque rupture of brain arteries. However, we cannot establish whether increased in situ MMP-9 activity in brain arteries is a primary event caused by weakening antioxidant defenses or whether it is a consequence of increased lesion formation. Increased plasma oxidative stress31,32 and low plasma antioxidant activity33 are seen during stroke in middle-aged men. Experiments evaluating whether interventions strengthening antioxidant defenses offer particular benefits to elderly subjects may resolve the question of whether increased oxidative stress contributes to increased atherogenesis and/or stroke. Although the present results support a causal role of oxidative stress in increased atherogenesis, it cannot be ruled out that the increase is the result rather than the cause of increased lesion formation. For example, it is conceivable that the progression of lesions disrupts protective anatomic features of intracranial arteries or that the generation of antioxidant enzymes is affected once lesions reach a certain stage.

Acknowledgments
This study is dedicated to the memory of Dr Fulvio Pinto (1916 to 1981) and was supported by grants 97/60% from the Ministero della Universitá e Ricerca Scientifica e Tecnologica (grant ISNIH, 33343/97) and from the National Heart, Lung, and Blood Institute (grant HL-56989).

References
An increasing amount of evidence suggests that oxidative mechanisms, in particular oxidative modification of LDL, are closely related to a number of inflammatory reactions, which seem essential for both initiation and progression of atherosclerosis. This process involves infiltration of inflammatory cells, intimal thickening, accumulation of extracellular matrix, fibrous cap formation, and angiogenesis. Whereas atherosclerotic plaques develop over decades, it is largely unknown what suddenly triggers an unstable plaque to rupture and cause clinical symptoms such as unstable angina, myocardial infarction, and stroke. The protective role of antioxidants such as vitamin E on atherosclerotic disease or clinical event rate is still unclear, but research in this area continues. The article by D’Armiento et al is part of the intense search for links between age, antioxidants, and atherogenesis.

The initiating step of atherogenesis is thought to be endothelial dysfunction, which may be caused by one or several factors, including elevated and oxidized LDL as well as free radicals caused by smoking, hypertension, and diabetes. These factors activate the endothelial cells, expressing adhesion molecules such as the vascular cell adhesion molecule (VCAM)-1 and promoting monocyte and T-lymphocyte infiltration of the intima. To minimize the damage caused by oxidation, the oxidized LDL particles are internalized by the activated monocytes, so-called macrophages, via surface scavenger receptors, thereby transforming them into foam cells. Macrophages produce cytokines and growth factors and induce smooth muscle cell proliferation, ultimately increasing atherosclerotic plaque size. The inflammatory process also involves upregulation and activation of matrix metalloproteinases (MMPs) in endogenous smooth muscle cells. Increased production of MMPs in the shoulder region of the plaque is thought to play a role in plaque instability and rupture through degradation of extracellular matrix and the fibrous cap, facilitation of migration through the endothelial cell layer and basement membrane, intimal thickening, and angiogenesis.

Supporting the oxidation theory outlined above, structurally different antioxidants such as vitamin E, probucol, butylated hydroxytoluene, and N,N’-diphenyl-phenylene diamine are known to both inhibit ex vivo LDL oxidation and reduce free radical formation by modified LDL and atherosclerosis in animals. However, in the clinical trials reported, vitamin E intake/supplementation reduced the incidence of myocardial infarction in patients with established coronary artery disease in the Cambridge Heart Antioxidant Study (CHAOS) trial, but not in the Alpha-Tocopherol Beta-Carotene (ATBC), Heart Outcomes Prevention Evaluation (HOPE), and Gruppo Italiano per lo Studio della Sopravvenza nell’ Infarto Miocardico (GISSI) trials. Furthermore, the outcomes of intervention trials with vitamin C in coronary heart disease are still disappointing. This indicates that our knowledge about links between lipoprotein oxidation and atherogenesis is incomplete.

In this autopsy study by D’Armiento et al, the authors pursue their original hypothesis that intracranial arteries possess a greater resistance than do extracranial arteries. This could be due to a better antioxidant protection of the intracranial arteries, which might also explain the later occurrence of atherogenesis in these vessels. The authors also describe the effect of aging on the extent of atherosclerosis and compare this to the activity of 4 important antioxidant enzymes (glutathione peroxidase, Mn and CuZn superoxide dismutase, and catalase) and the MMP-9 (gelatinase-2) in the walls of intracranial and extracranial arteries. The histomorphometric amount of atherosclerosis was found to increase linearly with age in the 4 different male age groups (premature fetuses, children, adults, and elderly) without known risk factors for atherosclerosis. At any age, the amount of atherosclerosis was 6- to 19-fold greater in extracranial than in intracranial arteries. Furthermore, the antioxidant activity decreased with age in the intracranial arteries. Moreover, MMP-9 expression and gelatinolytic activity increased with age and correlated with severity of intracranial atherosclerosis. The fact that levels of MMP-9 and LDL both increased with age and were colocalized in atherosclerotic lesions suggests that age-related overexpression of MMP-9 may play a role in plaque rupture. Supporting this hypothesis, MMP-9 activity was found to correlate with human carotid plaque instability. As these studies point out, the question of whether increased in situ MMP-9 activity and atherogenesis in cerebral arteries is a result of weakened antioxidant defenses or a consequence of increased lesion formation remains unanswered.

When these findings are implemented in future research, the role of age must not be underestimated. Intervention trials with antioxidants should include younger, less-diseased individuals than previously studied, since the protective effects against atherogenesis, cardiovascular disease, and events may be most profound in individuals aged 20 to 40 years or may need a longer period of time to exert protection. Such data are needed as valuable directions for targeting preventive therapy against complications to atherosclerosis, which remains the leading cause of death in the industrial world.

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Age-Related Effects on Atherogenesis and Scavenger Enzymes of Intracranial and Extracranial Arteries in Men Without Classic Risk Factors for Atherosclerosis
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