Original Contributions

ACE Inhibition With Perindopril and Atherogenesis-Induced Structural and Functional Changes in Minipig Arteries

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The effects of angiotensin-converting enzyme (ACE) inhibition on atherosclerosis-induced changes in arterial function are unknown, as well as whether they are coupled to improvements of structural alterations in the arterial wall. An atherogenic (A) diet and the ACE inhibitor perindopril (P) were given concomitantly for 4 months to seven adult Pitman-Moore minipigs (7 months of age; A+P animals), which were compared with seven A and seven control (C) animals. Perindopril, at a daily dose of 4 mg PO that is commonly used in the clinical setting, induced a continuous 70% inhibition of serum ACE activity. At the end of the study, the atherosclerosis-induced impairment of arterial flow was investigated via the hemodynamics and vascular rheology of hindlimb arteries in non-barbiturate-anesthetized pigs. Structural alterations were evaluated from the histopathology of lesions in the arterial tree (abdominal aorta, left interventricular coronary artery [LIVCA], and brachiocephalic trunk [BCT]), with particular attention given to the analysis of the structure and composition of aortic elastic fibers. Atherosclerosis impaired the function of both capacitance and resistance arteries. Blood pressure (BP) rose significantly because of increased hindlimb peripheral resistance (HPR) and aortic input impedance (Zc), although blood flow was not affected. Altered aortic stress and elastic responses revealed that the stiffness of the aorta was markedly increased because of increased wall tension and reduced viscoelasticity, the viscous component being blunted in the arterial wall. Perindopril significantly opposed these alterations by reducing BP, HPR, and Zc and by returning parietal stiffness values to C values by increasing aortic compliance. ACE inhibition prevented the alteration of both stress and elastic responses. Major fibroproliferative fatty lesions were observed in the aorta and LIVCA, while moderate fibrosclerotic lesions were found in the BCT. Computerized densitometric analysis of orcein-stained elastin showed that elastic laminae fragmentation was prominent in the abdominal aorta, less in the LIVCA, and moderate in the BCT. Furthermore, the elastin content was reduced in the atherosclerotic aorta, although this loss of elastin was not associated with changes in the biochemical nature of alkali-insoluble elastin. Perindopril significantly prevented the development of atherosclerosis in the abdominal aorta, LIVCA, and BCT by decreasing the cross-sectional area of lesions as well as the number of lipid-laden cells in the abdominal aorta and LIVCA. In the abdominal aorta, ACE inhibition significantly prevented the alteration of elastic laminae by specifically preventing elastolytic fragmentation of dense elastic laminae, but it did not modify elastin content. In conclusion, ACE inhibition with perindopril showed a significant preventive action on atherosclerosis-induced deleterious effects on vascular wall function and structure, especially by reducing fragmentation of aortic elastic laminae. (Arteriosclerosis and Thrombosis 1993;13:1125-1138)

KEY WORDS • atherosclerosis • elastin • hemodynamics • vascular rheology • angiotensin-converting enzyme • angiotensin-converting-enzyme inhibitors • perindopril • minipig

There is now considerable interest in the involvement of the renin-angiotensin system in the regulation of the function, structure, and growth of large and small vessels under either physiological or pathological conditions. Angiotensin II (Ang II) not only is a potent vasoconstrictor but also influences, at least partially by nonpressor mechanisms, the vascular growth3-8 and biosynthesis of connective tissue.9.10 Since it has been suggested that the renin-angiotensin system may play a direct role in the development of chronic vascular diseases,1,2 the action of angiotensin-converting-enzyme (ACE) inhibitors on atherogenesis is worthwhile considering. Blockade of plasma and tissue ACE11,12 could modify atherosclerosis development by affecting both Ang II- and non-Ang II-dependent effects, eg, those due to bradykinin and prostaglandins.8,13 The effects of ACE inhibitors on the development of atherosclerosis are documented in various animal mod-
The animals were handled in accordance with the guidelines for animal use provided by the INSERM U-278 Animal Care and Use Committee, which granted approval of this experimental protocol. Twenty-one male Pitman-Moore minipigs (CEGAV, Passais-la-Conception, France) (7 months of age; body weight, 28.9±4.2 kg, mean±SD) were kept together in ample housing (2.8×18 m, subdivided into three stalls for each of the different animal groups) with natural daylight and free access to water. After a 2-week acclimatization period, the minipigs were randomized into three groups (n=7 per group): control (C), untreated atherogenic (A), and perindopril-treated atherogenic (A+P) minipigs. Group C animals were given a standard diet (500 g/d, 1500 kcal/d) consisting of cereals, animal and vegetable proteins, and vitamins (breeding diet 127 from UAR, Villemoisson sur Orge, France; total lipid content of 3%). The A and A+P animals were fed a high-fat, high-cholesterol, high-protein diet, which was previously found to induce marked atheromatous lesions in Pitman-Moore minipigs.18,19,21 and which contained (by weight) cholesterol (2%), peanut oil (4%), butter (30%), sodium cholate (1%), choline chloride (1%), caseins (40%), sucrose, salt, and vitamin mixtures. The A and A+P animals were fed this diet (400 g/d, 2000 kcal/d) for 4 months. They underwent surgery as A and A+P matched pairs within 2 consecutive days and were interspersed with C animals. The slightly higher amount of calories consumed by A and A+P than C animals has not been reported to introduce bias when investigating the development of atherosclerosis.21

Blood Collection and Processing: Serum Metabolic Parameters

Blood samples were drawn in the morning, ie, 24 hours after the last treatment and feeding, from the vena cava of each pig while in dorsal recumbency. As previously reported, serum metabolic parameters were evaluated by using appropriate Boehringer Mannheim kits and Hitachi 717 systems.18,19 Similarly, cholesterol content in the low-density lipoproteins (LDLs) was calculated according to the Friedwald formula:

\[
\text{LDL Cholesterol} = \text{Total Cholesterol} - \text{High-Density Lipoprotein Cholesterol} - \text{Triglycerides/2.2}
\]

with results expressed in millimoles per liter.

Perindopril Administration, Assay of ACE Activity, and Perindoprilat Serum Level

In the present experimental protocol, perindopril dose and route of administration were directly derived from the clinical treatment protocols of human hypertension31: minipigs, in a manner independent of body weight, were given a constant 4-mg daily dose of perindopril as one tablet inserted within their food. C and A animals were similarly given a placebo-matched tablet. Minipig compliance to treatment, ie, swallowing of the tablet, was carefully checked. The photopletysmographic measurement (Finapress Instruments, Ohmeda Ltd, Stockholm, Sweden) of the tail blood pressure in three C animals revealed that a single 4-mg perindopril dose induced a slight (10±4%) fall in blood pressure 6 hours after tablet administration.

Perindoprilat, the active metabolite of perindopril, was assayed by radioimmunoassay in sera from A+P animals before and after 1, 2, and 3 months of the
Surgical Procedures: Measurements of Hemodynamics and Aortic Wall Rheology

Surgery was performed as previously described, with the following modifications. Minipigs were anesthetized with an intravenous urethane/chloralose mixture (25%:5% vol/vol, 1.5 mL·kg⁻¹ of body weight), which avoids depressive cardiorespiratory effects, and were ventilated (50 mL/kg) at their spontaneous respiratory frequency. After a midline laparotomy, a 3-cm segment of the abdominal aorta was freed, at an equal distance between the renal and lower mesenteric arteries. The external aortic diameter (D) and its variation during a cardiac cycle (ΔD) were monitored on an oscilloscope (Tektronix 5103N) and measured as the transit time of ultrasonic acoustic pulses traveling at a velocity of 1.58 mm·μs⁻¹ between pairs of 5-MHz piezoelectric crystals that were either glued onto opposing surfaces of the aorta or enclosed in a flexible probe inserted around the aorta. Pulsatile and mean aortic blood pressures (MABPs) and flows (Qm) were measured with a 6F micromanometer-tip catheter with a fluid-velocity sensor (Millar Instruments model SVPC-664A), which was positioned under fluoroscopic guidance at the site where the diameter was measured. The electrocardiogram (ECG) was recorded (Datascope 850 F) and amplified (differential amplifier, Tektronix model AM 502) on both the oscilloscope and the computer (see below). Heart rate (HR) was computerized. Analog records were saved on a digital tape recorder (Bio-logic DTR 1800) after having been digitized (analog-to-digital converter model AD 2821 F, Analog Devices, Inc.).

From MABP and Qm, the hindquarter peripheral resistance (HPR) was calculated as HPR=MABP/Qm.

\[ \sigma = \frac{2P(ab/R)^2}{(b-a)^2} \]

and

\[ \text{Einc} = 0.75 \frac{R \delta a}{\delta R} \]

where, as previously described, and \( a \) and \( b \) are the inner and outer radii, respectively; \( R \) is the midwall radius; and \( P \) is the aortic MABP. It should be emphasized that Einc refers to the elastic stiffness of the aortic wall per se and is not to be confused with the terms \( \Delta V/\Delta P \) or \( \Delta P/\Delta D \), which refer to the compliance and stiffness, respectively, of the aorta as a hollow structure.

Aortic wall thickness (ho) was calculated as \((b-a)\) and expressed as a percentage of the midwall radius (ho/R×100). From the mean internal section of the artery (S) and aortic stress (\( \sigma \)), the mean nonoscillatory potential energy (Wm) was calculated according to:

\[ \text{Wm} = 5 \frac{\delta a}{\delta S} \]

This energy reflects the total cardiac energy necessary to propagate pressure and flow waves along the arterial tree, but differs from Einc, which refers to the radial and stress variations during a cardiac cycle.

Histological and Morphometric Analysis of the Vasculature

Pathological changes of the elastic BCT, muscular LIVCA, and musculoskeletal abdominal aorta were evaluated in a 0.5-cm-long segment of the BCT at the common carotid bifurcation, a 0.5-cm-long segment of LIVCA sampled immediately after the circumflex artery bifurcation from the left coronary trunk, and a 1.5-cm-long segment of abdominal aorta located at middistance between the left renal artery and aortic trifurcation, respectively. Therefore, all segments were collected identically for all animals. Immediately after the animals were killed (20 mL of 10% KCl wt/vol IV), vessel segments were rinsed in ice-cold isotonic saline solution. The segments of aorta (further sectioned transversely into six pieces), coronary arteries, and superaortic arteries were fixed in Bouin’s liquid for 24 hours. Longitudinal and transverse 4-μm serial sections were obtained and stained alternatively with hematoxylin, cosin, and safranin for general observation, Masson’s green trichrome for collagen and connective tissue, and Darrow’s orcein for specific staining of elastic tissue, as previously described.

Computerized morphometric analysis of orcein-stained pathological slides was performed using an automatic morphometric video analyzer (SAMBA 2005, TITN-Alcatel, Grenoble, France). On each section on four opposite poles, a radial axis was positioned manually and defined as the intimal-medial thickness; this is referred to as L. Thereafter, a rectangle having a width of \( \frac{L}{12} \) was computerized and focused on the radial axis, allowing us to use a normalized area. Elastic fibers within the rectangular frame were investigated under standardized light intensity, with orcein staining normalized on an inverse scale from OD of 255 (ie, lumen) to an OD of 0 for the more intense elastin staining (ie, black). Preliminary investigations of slides revealed that according to their OD along this scale, elastic fibers elicited a two-peak distribution, thus dis-
criminating high- versus low-density fibers that corresponded to native versus split-disrupted fibers. The presence of elastin was therefore evaluated at two different thresholds along the OD scale: value 152 encompassed all the elastic fibers and defined high- and low-density elastin fibers, whereas only high-density elastic fibers were recorded at value 127. Pathological data for each pole of the vascular ring were averaged. The cross-sectional area of the atheromatous lesions encompassed all the elastic fibers and defined high- and low-density elastin fibers, whereas only high-density elastin fibers were recorded at value 127. Pathological data for each pole of the vascular ring were averaged. At prominent lesion sites, the depth of the lesion within the vascular wall was evaluated as the ratio of the number of split-disrupted to total elastic laminae. Observations of slides under the light microscope were based on a previously reported classification for the human aorta. The presence of lipid in the thickened intima was expressed as the intimal area where lipid-laden cells and/or foam cells were observed with respect to the overall intimal area. All histomorphometric analyses were blinded.

Analysis of Elastin From Aortic Vascular Walls

At surgery, a 3-cm segment of the abdominal aorta was collected, centered on its point of attachment to the renal arteries. The adherent connective tissue was carefully removed from the aortic wall. The dry weight of lyophilized aortic fragments was determined. Elastin was purified from 150 mg of aortic wall and powdered in liquid nitrogen according to Lansing et al.41 The final residue was lyophilized and weighed. The chemical composition of elastin was determined by evaluating the amino acid composition of the final protein residue after acid hydrolysis (6N HCl for 16 hours at 110°C) using a Beckman 118BL amino acid autoanalyzer according to Moore et al.42 This method also allowed us to determine the amount of desmosine and isodesmosine, which reflects the cross-linking index of elastin.

Statistical Analysis

Data are reported as mean±SD. All data from individual minipigs were computed and stored on STATGRAPHIC software. Statistical analysis was performed with an analysis of variance and a Mann-Whitney U test. A value of $P<.05$ was considered statistically significant.

### Table 1. Serum ACE Activity and Lipid Profiles in Minipigs

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=7)</th>
<th>Group A (n=7)</th>
<th>Group A+P (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE (IU/L)</td>
<td>696±75</td>
<td>642±49</td>
<td>747±78</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.9±0.3</td>
<td>0.9±0.3</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.9±0.1</td>
<td>2.1±0.2</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>LDL chol (mmol/L)</td>
<td>0.6±0.3</td>
<td>0.8±0.2</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>HDL chol (mmol/L)</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>0.8±0.3</td>
</tr>
</tbody>
</table>

Results are mean±SD for control (C), atheromatous (A), and perindopril-treated atheromatous (A+P) minipigs before (day 0) and at the end of the standard or atherogenic diet (ie, 4 months). ACE, angiotensin converting enzyme; LDL, low-density lipoprotein; chol, cholesterol; HDL, high-density lipoprotein.

*P<.01 compared with respective C value.

### Table 2. Hemodynamic, Rheologic, and Viscoelastic Parameters in the Aorta of Minipigs

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=7)</th>
<th>Group A (n=7)</th>
<th>Group A+P (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>101±10</td>
<td>104±9</td>
<td>98±8</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>114±6</td>
<td>153±8†</td>
<td>121±9‡</td>
</tr>
<tr>
<td>Qm (mL·min⁻¹)</td>
<td>244±33</td>
<td>203±28</td>
<td>222±16</td>
</tr>
<tr>
<td>HPR (mm Hg · mL⁻¹·min)</td>
<td>0.51±0.06</td>
<td>0.80±0.07‡</td>
<td>0.55±0.04§</td>
</tr>
<tr>
<td>Zc (mm Hg · mL⁻¹·min)</td>
<td>0.224±0.021</td>
<td>0.286±0.021*</td>
<td>0.234±0.012‡</td>
</tr>
<tr>
<td>R (cm)</td>
<td>0.305±0.016</td>
<td>0.330±0.014</td>
<td>0.316±0.014</td>
</tr>
<tr>
<td>ho/R (%)</td>
<td>20.42±1.52</td>
<td>20.74±1.50</td>
<td>21.90±1.99</td>
</tr>
<tr>
<td>Co [(mL·mm Hg⁻¹)×10⁻³]</td>
<td>143±22</td>
<td>65±6†</td>
<td>120±16‡</td>
</tr>
<tr>
<td>α (kN·m⁻²)</td>
<td>80±9</td>
<td>108±6*</td>
<td>86±8‡</td>
</tr>
<tr>
<td>Einc (kN·m⁻²)</td>
<td>2512±599</td>
<td>4917±818*</td>
<td>2550±301‡</td>
</tr>
<tr>
<td>AoS (mm Hg·cm⁻¹)</td>
<td>8112±1432</td>
<td>17.560±2269†</td>
<td>9551±1159§</td>
</tr>
<tr>
<td>Wm (kN·m⁻²)</td>
<td>1524±304</td>
<td>2663±469</td>
<td>1596±151‡</td>
</tr>
</tbody>
</table>

Parameters were measured or calculated in control (C), atheromatous (A), and perindopril-treated atheromatous (A+P) minipigs at the end of the standard or the atherogenic diet (ie, 4 months). HR, heart rate; MABP, mean arterial blood pressure; Qm, mean blood flow; HPR, hindlimb peripheral resistance; Zc, aortic input impedance; R, midwall radius; ho/R, wall thickness/midwall radius ratio; Co, aortic volume compliance; α, midwall aortic stress; Einc, incremental elastic modulus; AoS, aortic wall stiffness; Wm, mean nonoscillatory potential energy.

Group A significantly different from group C at *P<.05, †P<.01.

Group A+P significantly different from group A at $\S P<.05, \S P<.01$. Note that none of the values measured or calculated for the A+P group is significantly different from the corresponding values measured or calculated for group C.
Results
Control, Atherogenic Diet−, and ACE Inhibition−Induced Changes in Serum Metabolic Parameters and ACE Activity

All animals remained healthy throughout the treatment period. Atheromatous minipigs tolerated the atherogenic diet well and gained weight, as did the standard diet−fed C animals. Final weights were 38±5, 44±7, and 43±13 kg for C, A, and A+P groups, respectively. There were no significant changes in serum biochemistry (data not shown) with the exception of elevated cholesterol-related serum parameters in the animals fed the atherogenic diet, which revealed type IIa hyperlipoproteinemia (Table 1). Hypercholesterolemia stabilized after 1 month of feeding. Lipoprotein electrophoresis revealed that the LDL to HDL ratio increased in group A from 0.69±0.12 before feeding to 1.94±0.52 when hypercholesterolemia was maximal at 3 weeks and to 1.58±0.62 at the end of the protocol. No significant differences in cholesterol-related serum parameters were noted between perindopril-treated and untreated atheromatous animals (Table 1).

After 1, 2, and 3 months of perindopril administration, perindoprilat serum levels in A+P animals were found to be 6.5±1.5, 3.4±0.7, and 4.5±0.8 ng/mL, respectively. Serum ACE activity remained constant in placebo-treated C and A animals throughout the duration of the protocol, whereas ACE activity was inhibited by 70% in perindopril-treated minipigs (Table 1). ACE inhibition was achieved in A+P animals within 2 weeks of ACE inhibitor administration (data not shown).

Hemodynamics and Vascular Wall Elastic Properties in Atheromatous Minipigs: Effects of ACE Inhibition

Table 2 summarizes the effects of the atherogenic diet on the hemodynamics and aortic rheologic and viscoelastic parameters in the overall hindlimb vascular tree of group A minipigs compared with group C or group A+P minipigs. Compared with group C, atheromatous animals elicited a significant increase in MABP, HPR, and Zc, whereas Qm was reduced, but not significantly so. Such a significant rise in MABP without any significant change in Qm resulted from the significant increase in HPR. Atherosclerosis markedly affected the elastic behavior of capacitance vessels by reducing Co and increasing AoS, α, and Einc. The ho to R ratio in group A was unaffected, ho and R being simultaneously slightly increased. Wm, reflecting the stroke work, tended to be increased, although not significantly. HR was not affected by atherosclerosis.

Compared with untreated group A animals, perindopril treatment induced a significant decrease in
Fig 3. Left, Photomicrographs illustrating typical appearance of arterial vascular walls from atheromatous minipigs and histological consequences of angiotensin-converting enzyme inhibition in perindopril-treated animals. A and B show the fibroproliferative and lipid-laden lesions observed in the atheromatous abdominal aorta (Masson's trichrome, longitudinal section, original magnification ×200) and left interventricular coronary artery (LIVCA) (orcein, transverse section, original magnification ×225), respectively. In the aorta, note the appearance of most of the luminal elastic laminae, which, combined with vascular cell proliferation and lipid deposition, effected the intimalization of the media; in the LIVCA, the protruding character of the stenosis, bulging over the inner layers of the media, and the swelling of the endothelial cells are shown as well. In atheromatous minipigs, histological disorders characteristic of the brachiocephalic trunk were essentially fibroelastic (C, Masson's trichrome, transverse section, original magnification ×200). Between disrupted elastic laminae, the thickened space was filled with unorganized, amorphous fibroelastic materials. In the abdominal aorta from treated animals (D, Masson's trichrome, transverse section, original magnification ×100; E, orcein, transverse section, original magnification ×200), pathological alterations appeared as focal proliferative process and were restricted to the thickening and splitting of elastic laminae and to moderate lipid deposits. In the LIVCA from treated animals (F, orcein, transverse section, original magnification ×100), bulging of coronary lesions was moderate, giving the lesion a flattened appearance, with partially preserved parallelism of elastic laminae. Alterations of the brachiocephalic trunk in treated animals were found to be only moderate (G, Masson's trichrome, transverse section, original magnification ×200).

MABP, HPR, and Zc. In addition, Qm was slightly but nonsignificantly increased. Co was significantly higher in group A+P than in group A. Furthermore, α, Einc, AoS, and Wm were significantly reduced, and ho, R, and ho/R were unaffected by perindopril treatment. As a result, none of the measured or calculated values in group A+P were found to be significantly different from the corresponding values in group C.

Typical changes in the width of the hysteresis of the pressure-external aortic diameter loops reflected changes in the viscoelastic properties of the arterial wall (Fig 1). Compared with the situation in group C animals, the flattening of the hysteresis loop in group A animals illustrates that the amplitude of the viscoelastic response was significantly lowered in the latter, as a consequence of a significant decrease in both pulse pressure and pulse diameter. In group A+P, the loop was nearer to the control situation when compared with group A, thereby indicating that the elastic response was retained to a great extent in this group of animals. The diagrams of midwall aortic stress (σ) versus midwall radius (R), elastic modulus (Einc), and aortic compliance (Co) further support this view (Fig 2). The σ-Einc relationship demonstrated an important shift in both σ and Einc toward higher values in the A group. A similar shift was observed for the σ-R relationship, ie, the "stress response," clearly indicating an increase in vascular smooth muscle tension. Similarly, the "elastic response" represented by the σ-Co plot was highly diminished in group A animals because of a rise of σ values and a concomitant decrease of Co.

Histopathological Features of Atherosclerotic Vascular Lesions: Effects of ACE Inhibition

As a result of histological examination, the control animals were found to be not entirely free of lesions. The LIVCA and to a lesser extent the abdominal aorta of these animals exhibited few essentially fibroelastic vascular streaks (data not shown), typically resembling those of aging vessels.26,27 BCTs were found to be disease free.

In the A group, the abdominal aorta (Fig 3, A) and LIVCA (Fig 3, B) showed a pronounced fibroelastic disorder associated with marked lipid accumulations. Both aortic and coronary lesions were protruding within the luminal space, independent of lesion size. Aortic lesions showed both luminal and intravascular wall development of disease characteristic of medial intimalization53-54 (Fig 3, A), while coronary atherosclerotic lesions preferentially protruded into the vascular lumen, thus preserving the inner layers of the coronary media53-55 (Fig 3, B). At both arterial levels, numerous lipid-laden and foam cells were present within the intima and media, internal elastic laminae were often split and disrupted, and significant amounts of fragmented elastic fibers and collagen were observed. Reorientation of medial smooth muscle cells was commonly found associated with internal elastic lamina disruption in aortic lesions, thereby suggesting that spreading processes had occurred. On the other hand, the lesions in the BCT progressively deepened within the vascular wall (Fig 3, C). They were characterized by prominent scattering of elastic laminae and excessive amounts of abnormal fibrous components in the medial layer. Significant lipid deposition (<1% of the overall lesion) was observed within the lesions of the BCT.

Atheromatous lesions were still observed in the vessels of perindopril-treated minipigs but to a lesser extent than in animals from the A group. In the abdominal aorta of A+P animals (Fig 3, D and E), a protruding fibrofatty thickened intima was scarcely detected. The merging of underlying elastic laminae occurred occasionally, thereby preserving at least in part the stacked appearance of elastic laminae (Fig 3, E). However, disruption and splitting of the elastic laminae still occurred within focal areas of fibroproliferation and lipid deposition (Fig 3, D). Similarly, the stacked appearance of the elastic laminae was preserved in LIVCAs from treated animals, and bulging of the coronary stenosis was restrained, leading to a flattened appearance of atherosclerotic lesions (Fig 3, F). In the BCTs of treated animals, thickened elastic laminae were still observed close to the lumen of the vessels, whereas disruption of fibers and accumulation of fibrous material between elastic laminae were encountered only occasionally (Fig 3, G).

Computerized morphometric analysis of the lesions provides quantification of atherosclerosis-induced histological changes and the effects of ACE-inhibition thereon (Fig 4). In elastic (ie, BCT), musculoelastic (ie, abdominal aorta), and muscular (ie, LIVCA) arteries of untreated animals, significant atherosclerotic lesions were encountered, as well as alterations in the organization of elastic laminae. Accumulation of lipids within the thickened intima was found at all three arterial sites.
when compared with controls (Fig 4). ACE inhibition induced a marked decrease in both the extent of lesions, as revealed by the lesion cross-sectional area, and alterations of fibroelastic structures, ie, intraparietal depth of lesions, in all three investigated sites. The presence of lipids in intimal thickening in both the subrenal aorta and LIVCA was largely and significantly reduced by ACE inhibition.

Elastin in Vascular Walls: Effects of ACE Inhibition

The typical amino acid composition of alkali-insoluble elastin extracted from the subrenal aorta demonstrated the purity of the final material (Table 3). The molecular composition of elastin in the residual elastic structures of the aorta was unaffected by atherogenesis or perindopril treatment, since there were no significant changes in elastin composition between group C, A, and A+P ani-
TABLE 3. Amino Acid Composition of Elastin in Minipig Subrenal Aortas

<table>
<thead>
<tr>
<th>Group</th>
<th>Asp+Asn</th>
<th>Hydroxy-Pro</th>
<th>Thr</th>
<th>Ser</th>
<th>Gln+Glu</th>
<th>Pro</th>
<th>Gly</th>
<th>Ala</th>
<th>Val</th>
<th>Cys</th>
<th>Ile</th>
<th>Leu</th>
<th>Tyr</th>
<th>Phe</th>
<th>Ide</th>
<th>Des</th>
<th>Lys</th>
<th>His</th>
<th>Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n=7)</td>
<td>6.7±0.8</td>
<td>16.3±1.9</td>
<td>11.9±0.9</td>
<td>9.4±0.9</td>
<td>18.5±1.6</td>
<td>91.8±5.7</td>
<td>350±7.7</td>
<td>252±6.5</td>
<td>129±4.8</td>
<td>0.44±0.2</td>
<td>10.8±0.5</td>
<td>43.6±0.8</td>
<td>13.7±0.6</td>
<td>29.4±1.3</td>
<td>0.67±0.09</td>
<td>1.28±0.07</td>
<td>5.7±0.4</td>
<td>1.7±0.1</td>
<td>6.3±1.2</td>
</tr>
<tr>
<td>A (n=7)</td>
<td>7.1±1.2</td>
<td>15.6±3.2</td>
<td>11.4±1.2</td>
<td>9.1±1.1</td>
<td>18.8±2.3</td>
<td>96.4±7.4</td>
<td>361±34.2</td>
<td>236±23.5</td>
<td>131±18.8</td>
<td>0.49±0.35</td>
<td>10.8±0.9</td>
<td>43.9±3.9</td>
<td>14.0±1.7</td>
<td>28.8±3.4</td>
<td>0.64±0.06</td>
<td>1.27±0.01</td>
<td>5.5±1.1</td>
<td>1.8±0.4</td>
<td>5.4±1.7</td>
</tr>
<tr>
<td>A+P (n=7)</td>
<td>7.1±0.9</td>
<td>15.3±2.3</td>
<td>11.8±0.8</td>
<td>9.3±0.7</td>
<td>18.7±1.7</td>
<td>92.9±7.3</td>
<td>356±7.4</td>
<td>250±9.1</td>
<td>127±6.3</td>
<td>0.21±0.2</td>
<td>10.8±0.7</td>
<td>43.9±1.0</td>
<td>13.8±0.8</td>
<td>28.3±2.8</td>
<td>0.70±0.08</td>
<td>1.35±0.09</td>
<td>5.0±1.4</td>
<td>1.7±0.2</td>
<td>5.8±1.9</td>
</tr>
</tbody>
</table>

Results are mean±SD in residues per 1000 from control (C), atheromatous (A), and perindopril-treated atheromatous (A+P) minipigs at the end of the standard or atherogenic diet (ie, 4 months). Des, desmosine; Ide, isodesmosine.

This was especially true for the content of the cross-linked amino acids desmosine and isodesmosine, which was similar in the three groups (Table 3). When compared with C animals, dry weight measurements of elastin residues showed a marked decrease of aortic elastin in A and A+P animals (Fig 5). In this setting, no significant difference was noted between A and A+P animals.

Investigation of the microscopic organization of elastic fibers by computerized morphometric analysis revealed that the number of elastic fibers was decreased within the vascular wall in each of the three vascular sites of atheromatous minipigs, although the intensity of elastolytic processes varied widely among these vascular sites (Fig 4). Disappearance of elastic fibers was a major consequence of atherogenesis in the musculoelastic abdominal aorta and the muscular LIVCA, whereas the elastic BCT was less affected by this process: the overall presence of elastic fibers was reduced by 41%, 34%, and 11% in the abdominal aorta, LIVCA, and BCT, respectively. Fig 4 also reveals qualitative changes in elastic fibers due to vascular elastolysis: the amount of high-density elastic fibers decreased by 80%, 55%, and 23% in the abdominal aorta, LIVCA, and BCT, respectively, in atheromatous animals when compared with group C. In contrast, no significant difference in the presence of low-density elastic fibers was noted between the three groups (data not shown).

Perindopril significantly reduced by 35% overall the disappearance of elastic fibers in the abdominal aorta (Fig 4). On the other hand, perindopril did not induce significant changes in elastic fiber integrity in the LIVCA or BCT, which were otherwise only slightly affected by elastolysis (Fig 4). In addition, ACE inhibition significantly protected the abdominal aorta from the atherogenesis-induced splitting and disruption of elastic laminae, because when compared with C animals, the presence of high-density elastic fibers was only lowered by 57% in A+P animals, whereas it was lowered by 80% in untreated animals.

Discussion

Hemodynamics, Vascular Rheology, and Histopathology of Atherosclerotic Minipigs

Previous investigations of the potential preventive effects of ACE inhibition against atherogenesis and differences among various treatment approaches have been carried out in rabbits and cynomolgus monkeys.14-17 These models have recognized the limitations in extrapolating conclusions from functional and structural studies of vessels in animals to humans because of morphological, metabolic, hemodynamic, rheologic, and histological differences. Blood pressure and flow waves (travel and reflection and regional pulse propagation characteristics) in baboon and rabbit aortas as well as high heart rates and very high systemic vascular resistances (normalized for body weight) in these animals are markedly different from normal middle-aged humans.46-48 In addition, rabbits fail to develop atherosclerosis naturally, although a high-cholesterol diet (with or without endothelial denudation) may produce intimal thickening without involvement of the media in the abdominal aorta.24,35,39,43,49 Our study used Pitman-Moore minipigs, animals that spontaneously develop atherosclerosis22,23,26,27 and whose platelet-coagulation system is more closely related to that of humans.49 In the present study, we found the hemodynamics and viscoelastic properties of control minipigs to be closely related to those of humans. Specifically, the counterclockwise direction of the hysteresis loop of the blood pressure-aortic diameter relationship shows a delay between the change in aortic diameter during the initial increase in blood pressure and a significant
width of the hysteresis loop when aortic diameter remains constant during both early increases and decreases of blood pressure. These features illustrate the viscous response of the normal arterial wall described in humans.\textsuperscript{20,47,51,52}

In our study, minipigs fed the atherogenic diet remained perfectly healthy but developed type IIa hyperlipoproteinemia with cholesterol levels relevant to the human situation (10 to 15 mmol/L).\textsuperscript{53} Their hypertensive state characterized by increased blood pressure, peripheral resistance, and impedance without change in hindlimb blood flow illustrates that resistance arteries smaller than 0.4 mm in diameter were affected by atherosclerosis, although it is well known that they remain free of atherosclerotic lesions.\textsuperscript{45} In agreement with previous findings that hypercholesterolemia alters the vascular reactivity of small resistance arteries,\textsuperscript{54,55} high levels of circulating cholesterol and lipids incorporated into specific lipoproteins may presumably be a sufficient pathological stimulus to increase peripheral resistance and impedance.

In relation to capacitance vessel function, analysis of aortic wall rheology revealed significant decreases in both pulse pressure and pulse diameter originating from a marked decrease in vascular volume compliance. In addition, the minipigs showed a parallel increase of aortic midwall radius and wall thickness, which resulted in an unchanged h0 to R ratio. Changes in the elastic modulus and the shape of the pressure-diameter hysteresis loop of the aorta, as well as altered stress and elastic responses, revealed the increased stiffness of the abdominal aorta due to both increased vascular tension and failure of parietal viscoelastic properties. In particular, the loss of inertia within the diameter response to early changes in systolic blood pressure brings the vascular wall closer to the configuration of a hollow elastic tube, in which the viscous component is markedly altered while the intrinsic elastic component becomes dominant.\textsuperscript{37,38} It is known that the vascular response to tension (which corresponds to blood pressure in the capacitance vessel) includes two components: a quick elastic one and one that accounts for delayed relaxation.\textsuperscript{51,56} In our study, we have demonstrated that both components are altered by atherosclerosis, especially the viscous component, thereby providing evidence for the impairment of capacitance arteries. Since the viscoelastic behavior of capacitance arteries was investigated in the aorta, therefore measurements made at the entry of the hindlimb vascular bed evaluated the maximal magnitude of the viscoelastic properties of the capacitance arteries, which in parallel with the number of elastic laminae, progressively decrease from the entry of the vascular bed to the small resistance arteries. It is likely, although it remains to be clearly established, that the atherosclerosis-induced alterations observed in the aorta reflected those of the smaller capacitance arteries, proportionally to their localization along the arterial tree.

In adult minipigs, the vessels only had aging-related adaptive intimal thickening and fibroproliferative lesions with little lipid accumulation in the abdominal aorta.\textsuperscript{26,57} Histopathological analysis of vascular atherosclerotic lesions revealed fibroproliferative lesions in the BCT and fibroproliferative diseases with marked lipid accumulations in the abdominal aorta and LIVCA, both having a stenotic character, although the former had in-depth parietal development and the latter preserved the inner layers of the media. These three arterial sites were selected when designing the present study because (1) they are of fundamental clinical interest with respect to stroke, arteriosclerosis obliterans of lower limbs, and myocardial infarction and (2) they are representative of musculoelastic, muscular, and elastic arteries, respectively. It is worth noting that the lesions found in the minipigs of the present study were similar to the specific organization and nature of atherosclerotic lesions found in similar regions of human arteries.\textsuperscript{44,51,57}

Vascular Elastin and Elastic Fibers in Atherosclerotic Minipigs

In the present study, atherosclerosis development was accompanied by a significant loss of elastin content in the subrenal aorta of minipigs, but the amino acid composition of the residual alkali-insoluble elastin remained unchanged. The elastic fibers of the media consist of a microfibrillar component that first appears in the extracellular matrix and acts as a framework on which an amorphous component, elastin, an unusually stable component of mature and healthy elastic tissues, is deposited and insolubilized, specifically alkali insolubilized.\textsuperscript{44,57,58} In contrast, the precursor to the elastic component of the elastic fiber (tropoelastin) is alkali soluble. The usually very low rate of elastin turnover is accelerated under a variety of pathological conditions, such as pulmonary emphysema and atherosclerosis; elastin is then subjected to proteolytic degradation, leading to alkali-soluble elastin degradation products, de novo synthesis of abnormal elastin, and overall lowering of tissular elastin content.\textsuperscript{41,58,59} Abnormal elastin associated with the increased elastolytic process in advanced atherosclerotic lesions of the human aorta contains increased amounts of polar amino acids as well as reduced amounts of cross-linked desmosine and isodesmosine.\textsuperscript{59,60} In contrast, in the present study loss of elastin in the abdominal aorta was not found to be associated with a detectable change in the amino acid composition of residual elastin. This suggests that in our experimental model, the atherosclerosis-induced increased elastolytic process occurred without accelerated synthesis of abnormal elastin. However, synthesis of such abnormal elastin may be a slow process that becomes detectable in advanced atherosclerotic lesions only.

Computerized morphometric analysis of changes in the architecture and organization of orcein-stained elastic fibers revealed that elastic fiber fragmentation was different from region to region of the arterial tree: it was prominent in the abdominal aorta and occurred to a lesser extent in the LIVCA, whereas it remained moderate in the BCT. In the abdominal aorta, elastic fiber fragmentation resulted in an 80% disappearance of high-density fibers, whereas the overall elastin weight content was reduced by 25%. During atherogenesis in minipigs, elastic fiber fragmentation was therefore more pronounced than alkali-insoluble elastin disappearance. Accordingly, early elastolysis may induce prominent elastic fiber fragmentation, possibly amplified by concomitant lipid deposition, which has been shown to enhance susceptibility to elastases and/or release of nonspecific elastolytic activities from further infiltrated blood cells.\textsuperscript{59-61}
Relation Between Structural and Functional Atherosclerosis-Induced Changes

In the present study, the link between structural and functional changes was assessed by studying simultaneous alterations in elastin/elastic fiber organization and hemodynamics/vascular rheology. Fragmentation and splitting of the elastic fibers was a constant feature of atherosclerosis development in the vascular walls of atherosclerotic minipigs. Elastic fibers and connective tissue from a stress-tolerant network that facilitates the distribution of forces generated in the vessels. However, elastin plays a prominent role in vascular wall function. Collagen is much stiffer than elastin, the elastic modulus at 100% elongation in collagen being 350 times that in elastin.9,52 In the physiological range of blood pressure and parietal strain, the elastic fibers account predominantly for the wall response to the distending blood pressure and permit much of the necessary elastic recoil of the aorta against the pulse pressure.20 Changes in elastin presence and orientation have been shown to structurally affect the elastic behavior of the arterial wall more than similar changes in collagen.52,62 A pathological basis for altered elastic properties of the aorta has been reported in experimental animals with atherosclerosis: increased stiffness of the artery might be attributed to increased thickness of the intima, severity of atherosclerosis, and decreased vasa vasorum flow.52,62 Our results strongly support the view that the marked atherogenesis-induced fragmentation of dense elastic fibers, and to a lesser extent, insoluble elastin disappearance and slight increase of the wall thickness, progressively transformed the capacitance arteries into dense, elastic, hollow tubes by increasing arterial stress response (due to an increased vascular wall tension) and by decreasing arterial elastic response (due to a loss of viscoelastic properties of the wall, the viscous component being blunted).

Effects of ACE Inhibition on Atherosclerosis-Induced Changes

The low daily perindopril dose used in this study was selected to provide continuous ACE inhibition while keeping an ACE inhibitor dosage schedule that might be used in the clinical setting. Throughout the study, the treated minipigs had both perindopril plasma levels and serum ACE inhibition similar to those usually encountered in perindopril-treated patients.30 Furthermore, a significant blood pressure reduction was noted in treated atherosclerotic animals. Under similar circumstances in humans and animals, perindopril has the capacity to displace Ang I from ACE binding sites and to inhibit tissue and plasma ACE activity.11,31 Although the inhibition of plasma ACE does not reflect quite closely the inhibition of the Ang I to Ang II conversion,63 it is nevertheless likely that at least some inhibition of tissue (ie, vascular) ACE occurred in the perindopril-treated minipigs.

In the present study, significant differences in atherosclerosis-induced alterations of hemodynamics, wall rheology, histopathology, and elastic fiber organization were observed in the perindopril group, therefore favoring the use of ACE inhibitor therapy.

ACE inhibition prevented the deleterious hemodynamic effects of atherosclerosis without having addi-

It is known that a decrease in blood pressure per se modifies the function of large capacitance arteries as a result of changes in the geometry and viscoelastic properties of the arterial wall. According to the experiments of Hallock and Benson,69 the decrease in blood pressure is accompanied by a passive decrease in arterial diameter and volume, an increase in arterial compliance, and a proportional decrease in systolic and diastolic pressures. We report here that in minipigs fed an atherogenic diet, ACE inhibition decreased vascular wall tension and preserved the aortic wall from alterations of its viscoelastic properties, whereas both mid-wall radius and the ho to R ratio remained equal in the aortas of A and A+P animals. These findings imply that the beneficial effect of ACE inhibition in preventing the atherosclerosis-induced alterations of vascular wall function resulted from a specific action(s) of perindopril on the aortic wall instead of passive parietal improvements due to an action on the small resistance vessels and subsequent blood pressure lowering. This view is further supported by previously reported studies comparing the effects of ACE inhibitors with other vasodilating agents (eg, hydralazine), which were devoid of antiproliferative properties, although they lowered blood pressure (see Reference 17 for a review).

ACE inhibition slowed the overall histological development of atherosclerosis in muscular, musculoelastic, and elastic arteries of minipigs. Our results obtained in minipigs therefore confirm those obtained in a nonhuman primate model,16 but they differ from those obtained in cholesterol-fed rabbits14 and WHHL rabbits,15 in which beneficial effects of ACE inhibition on atherosclerosis development were either absent or limited to the thoracic aorta. As emphasized in a recent report on the effects of ACE inhibition on the neointimal hyperplasia responses to balloon injury, it is likely that the response to ACE inhibitor treatment may be species dependent64 and vessel specific as well. In addition, that report pointed out the prominent role of the choice of the daily dosage of ACE inhibitor when evaluating its pharmacological properties; indeed, compared with untreated animals, the use of a high-dose ACE inhibitor amplified neointimal formation after balloon injury.64 In an attempt to extrapolate our present results to the human situation, it is therefore worth noting that a therapeutic dose of perindopril showed beneficial effects in an atherosclerosis animal model having structural and functional characteristics similar to those found in human atherosclerosis.
Although the mechanisms accounting for the beneficial effect of ACE inhibitor against atherogenesis are not clearly understood, it is generally thought that the drug acts on atherosclerosis progression by blocking one or several uncontrolled mechanisms in the autocrine and paracrine functions that the renin-angiotensin system exerts on the arterial wall.\textsuperscript{1-10,64} In this setting, increasing interest has recently focused on kinins as potential mediators for the effects of ACE inhibitors, since they prevent kinin degradation by inhibition of kininase II, and in rats, a specific bradykinin B2-receptor antagonist attenuates the antihypertensive effects of ACE inhibitors\textsuperscript{66} and partly prevents the antiproliferative properties of ACE inhibitors in an experimental model of endothelial denudation.\textsuperscript{67}

The mechanisms of ACE inhibition effects on atherosclerosis-induced elastic fiber alterations remain unknown. However, differences in the topography, nature, and degree of alteration between atherosclerotic arterial sites may explain the significant action of perindopril reported in the abdominal aorta, whereas such effect was observed neither in the coronary artery nor in the BCT. Specifically, perindopril's effects were significant at the aortic site, where most of the dense, constitutive elastic fibers were affected and where a complex fibrotic, proliferative, and fatty lesion was observed associated with simultaneous in-depth (in contrast to LIVCA) and protruding (in contrast to BCT) development of the disease.

Destruction of medial elastin, an important mechanism underlying the sclerosis component of atherosclerosis, is associated in advanced lesions with increased collagen deposition and compositional changes in these fibrous proteins.\textsuperscript{64} The unchanged amino acid composition of residual elastin and reduced elastin content (by weight) demonstrate that the minipigs of the present study did not develop advanced atherosclerotic lesions, as confirmed by microscopic observations. These results further demonstrate that fragmentation and splitting of elastic fibers resulted from elastolytic activities rather than from a deficient assembly of new elastin fibers. The origin and nature of elastolytic enzymes involved in the degradation of elastic fibers in atherosclerotic lesions are not clearly understood. Medial elastin degradation cannot be attributed to either α\textsubscript{1}-antitrypsin deficiency or serum elastase until a fissure or an acute inflammatory lesion has occurred.\textsuperscript{59} The media of the stenotic atherosclerotic on aorta released a band (molecular weight of 30 KD) of elastolytic activity having a component immunologically identical with human leukocyte elastase. However, specific inhibitors of leukocyte elastase provide incomplete inhibition of vascular elastolytic activity, suggesting that other proteolytic activities are synergistic with elastase(s) and promote elastin degradation in vivo.\textsuperscript{59} In addition, release of activatable elastolytic enzymes from vascular cell lysosomes may either be induced by stretch or result from cell death.\textsuperscript{59,68} Finally, it is highly conceivable that the continuous activation of endogenous vascular elastases during atherogenesis, as well as the changes in elastin organization (ie, a close association of lipid deposits with elastin), result in enhanced susceptibility to elastases.\textsuperscript{61} In agreement with previous studies that have shown that elastin and collagen contents are lower and elastase and collagenase activities higher in patients with atherosclerosis,\textsuperscript{68} our finding of reduced elastic fiber degradation and lipid accumulation in the vascular wall of perindopril-treated minipigs failed to highlight the debatable question about whether these changes are the cause or the result of atherosclerosis development.

The mechanisms accounting for the preventive effects of ACE inhibition against elastic fiber degradation are unknown but might be related to the inhibition of plasma and/or tissue Ang II effects on the structural component of the vascular wall.\textsuperscript{9,10} Alternatively, perindopril, an inhibitor of proteolytic ACE, may inhibit one or several elastolytic enzymes. In this setting, it may be of interest to investigate the possible inhibitory action that ACE inhibitors may have on the various elastolytic activities found in both normal and atherosclerotic arteries.

The preventive effects of ACE inhibition on elastin fiber degradation are directly associated with the prevention of alterations of vascular wall function, at least in the hindlimb vascular bed. Once elastin is synthesized and stabilized by cross links, it displays low rates of turnover compared with other extracellular matrix proteins (years versus days or weeks).\textsuperscript{41,58,59} This elastin-specific feature takes on considerable importance for the vascular wall rheology of atherosclerotic vessels, since the elastic fibers subjected to degradation are not resynthesized in a normal manner, when then provides fibers that are inappropriate for normal function. Atherosclerotic lesions associated with clinical consequences have a major fibrous component, and the majority of sclerotic vessels have markedly affected wall rheology.\textsuperscript{68} Therefore, it is likely that the preventive effects of ACE inhibition on elastic fiber alterations will have major functional consequences that favor arterial blood circulation.

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ACE inhibition with perindopril and atherogenesis-induced structural and functional changes in minipig arteries.

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