Differential Effect of Experimental Hypertension and Hypercholesterolemia on Adventitial Remodeling

Joerg Herrmann, Saquib Samee, Alejandro Chade, Martin Rodriguez Porcel, Lilach O. Lerman, Amir Lerman

Objective—Intima-media remodeling, as frequently assessed by changes in the external elastic lamina-to-lumen area (EELLA), is well-described in coronary artery disease in contrast to adventitial remodeling, especially in the early disease stage.

Method and Results—Female domestic pigs were randomized to one of the following 12-week treatment groups: normal diet (N; n=6), high-cholesterol diet (HC; n=6), or renovascular hypertension (HT; n=4). Low-density lipoprotein (LDL) cholesterol serum concentration was higher in HC than in N and HT (395.5±106 versus 38.6±14 and 37.2±6.8 mg/dL; P<0.05 for both). Mean arterial pressure was higher in HT than in N and HC (141.3±21 versus 107.4±8.9 and 109.4±7.8 mm Hg; P<0.05 for both). EELLA ratio, as assessed by morphometry, was similar in N, HC, and HTN (1.03±0.32 versus 0.95±0.29 and 1.01±0.09; P<0.05 for both). Coronary vasorum density, as assessed by 3-dimensional micro-computed tomography, was higher in HC than in N and HT (3.4±1.0 versus 1.9±0.3 and 2.0±1.2; P<0.05 for both). In contrast, immunostaining showed a higher collagen III content and the presence of adventitial myofibroblasts in HT compared with N and HC.

Conclusions—The current study suggests that adventitial remodeling precedes intima and media remodeling of coronary arteries early after exposure to hypercholesterolemia and hypertension, with distinct qualitative differences between them. (Arterioscler Thromb Vasc Biol. 2005;25:447-453.)

Key Words: angiogenesis ■ oxidant stress ■ atherosclerosis ■ growth factors

Coronary artery remodeling has been characterized as an active process of structural changes in coronary artery disease, involving both vascular and adventitial components of the vascular wall, and is commonly assessed by the change in the external elastic lamina-to-lumen area (EELLA). Adventitial remodeling has been reported mainly in mechanical injury to the vascular wall and is marked, at least in part, by activation and transformation of fibroblasts into myofibroblasts, which can be referred to as vimentin/alpha smooth muscle cell (α-SMC) actin-positive cells. Whether this holds true to functional injury of the vascular wall by exposure to cardiovascular risk factors such as hypercholesterolemia and hypertension remains to be defined.

Experimental hypercholesterolemia and hypertension have been associated with metabolic and functional alterations, resembling early-stage cardiovascular risk factor exposure-related human conditions. Their initial effect on the vascular wall differs in the sense that hypercholesterolemia causes accumulation and oxidation of lipid particles in the subintimal space, whereas hypertension exerts a hemodynamic stimulus along with increase in endogenous oxidative stress. These mechanisms may exert different effects on various components of the vascular wall, and related experimental studies may be helpful to gain insight on the differential effect of cardiovascular risk factors on the alteration of the adventitial component of the vascular wall.

The current study was therefore designed to test the hypothesis that alterations in the structure of the adventitia occur in both experimental hypercholesterolemia and hypertension, yet with qualitative differences between them.

Methods

Animals

Female domestic pigs, 3 months of age (Pork Partners, Stewartville, Minn and Larson Products, Sargeant, Minn), were randomized to one of the following 12-week treatment groups: normal diet (N; n=6), high-cholesterol diet (HC; n=6) or renovascular hypertension (HT; n=4). Body weight, serum lipids, and blood pressure were determined weekly. Low-density lipoprotein (LDL) cholesterol serum concentration was higher in HC than in N and HT (395.5±106 versus 38.6±14 and 37.2±6.8 mg/dL; P<0.05 for both). Mean arterial pressure was higher in HT than in N and HC (141.3±21 versus 107.4±8.9 and 109.4±7.8 mm Hg; P<0.05 for both). EELLA ratio, as assessed by morphometry, was similar in N, HC, and HTN (1.03±0.32 versus 0.95±0.29 and 1.01±0.09; P<0.05 for both). Coronary vasorum density, as assessed by 3-dimensional micro-computed tomography, was higher in HC than in N and HT (3.4±1.0 versus 1.9±0.3 and 2.0±1.2; P<0.05 for both). In contrast, immunostaining showed a higher collagen III content and the presence of adventitial myofibroblasts in HT compared with N and HC.
MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 0.026 calcium ethylenediaminetetraacetic acid, and 11.1 glucose. All procedures were designed in accordance with the National Institutes of Health Guidelines and were approved by the Mayo Foundation Institutional Animal Care and Use Committee.

Microscopic Computed Tomography

After the removal of the heart, the left anterior descending coronary artery was prepared for 3-dimensional micro-computed tomography imaging as previously described. The computerized reconstruction algorithm yielded an average of 500 slices per coronary artery segment with a matrix of 42-µm cubic voxels and a 16-bit gray scale. All reconstructed image analyses were performed using the Analyze software (version 5.0; Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn.). Cross-sections were obtained at 1-mm intervals, excluding branching point areas and cutting artifact at the very ends of the specimen. In general, 5 to 12 cross-sections were analyzed per specimen, yielding the following study parameters: vessel wall and vasa vasorum (VV) area, VV count, VV density (ie, VV count related to VV area), mean diameter of first- and second-order VV, and ratio of the number of first- and second-order VV. On all cross-sections, further spatial stratification into 2 epicardial (E) and 2 myocardial (M) quadrants was made according to anatomic orientation.

Histological and Morphometric Analyses

Hematoxylin and eosin staining was performed according to standard techniques. Using a digital image system (Nikon DXM 1200), morphometric analyses on hematoxylin and eosin-stained slides were performed for the assessment of lumen area, internal elastic lamina area, and external elastic lamina (EEL) area. Intima area was calculated as internal elastic lamina area minus lumen area, and media area was calculated as EEL area minus internal elastic lamina area. The adventitia area was defined as the area between the EEL and the outer borders of the tissue specimen on the histology slides.

Immunohistochemistry

As described before, after deparaffinization in xylene and rehydration in 100%, 95%, and 70% ethanol, coronary slides were incubated with an equimolar solution of MeOH/H₂O₂ to block endogenous tissue peroxidase. In case of α-SMC actin, vimentin, and transforming growth factor (TGF)-β, antigen retrieval was made by incubation in 1 mol/L citrate acid solution. After protein L blocking, slides were incubated at 4°C overnight with the following primary antibodies and dilutions: anti-human α-SMC actin (1:100; Dako Corp, Carpinteria, Calif), anti-porcine vimentin (1:100; Dako), anti-porcine TGF-β (1:500; B飯店, Irvine, Calif), and anti-porcine endothelin-1 (ET-1) (1:100; Peninsula Laboratories, San Carlos, Calif), anti-human angiotensin-II (AngII) (1:500; Peninsula), and anti-human vascular endothelial growth factor (VEGF) (1:100; Santa Cruz Biotechnology, Santa Cruz, Calif). Detection and visualization of primary antibodies were subsequently performed by use of the EnVision kit (Dako), or in case of ET-1 and AngII by the use of specialized immunohistochemistry staining kits (S-4004 and S-4000; Peninsula) in peroxidase-labeling technique with 3,3-diaminobenzidine tetra-hydrochloride or 3-Amino-9-ethylcarbazole as chromogen.

For collagen III, antigen retrieval was made by incubation in 1 mg/mL trypsin (Sigma, St. Louis, Mo) for 15 minutes at room temperature, followed by blocking with casein (Vector Laboratories Inc, Burlingame, Calif) for 1 hour at room temperature. Subsequently, slides were incubated with an anti-collagen III antibody (Southern Biotechnology Associates, Inc, Birmingham, Ala) at a dilution of 1:20 for 1 hour at room temperature. Anti-goat IgG-HRP (Santa Cruz Biotechnology) was used as secondary antibody at a dilution of 1:20 for 30 minutes at room temperature. Vector Nova red substrate was used as the chromogen. Incubation with an unspecified isotype antibody served as a control for the specificity of immunoreactivity. All sections were counterstained with hematoxylin. Standard trichrome staining was used for the assessment of total collagen content.

Immunostaining and trichrome staining was quantified in 10 to 20 fields by use of a computer-aided image analysis program (Meta Imaging Series 4.6; MetaMorph, Universal Imaging Corporation, Downingtown, Pa) and expressed as percentage of staining of total surface area, with subsequent averaging of the results from all fields.

Immunoblotting

As described before, after removal of the heart, coronary arteries were snap-frozen in liquid nitrogen and stored at −80°C until further processing. All tissues (n=3 for HC and n=4 for HT) were homogenized using a tissue homogenizer and a lysis buffer of the following composition: 50 nM Tris HCl, pH 8.0, 150 mmol/L NaCl, 0.02% sodium azide, 0.1% SDS, PMSF 100 μg/mL, Aprotinin 1 μg/mL, 1% NP-40, and 0.5% sodium deoxycholate. The lysate was analyzed for protein content using a Bradford assay (Bio-Rad, Calif), and equal amounts of protein were resolved under reducing conditions on an 8% SDS-polyacrylamide gel. Immunoblotting was performed using an anti-HiP11 antibody (Santa Cruz Biotechnology Inc, Santa Cruz, Calif) at a dilution of 1:200 in a nonfat milk/Tris buffer. The polyvinylidene fluoride membrane was subsequently probed with a secondary anti-mouse antibody conjugated to horse-radish peroxidase (Amersham Biosciences Corp, Piscataway, NJ; dilution of 1:2000), developed with chemiluminescence (Pierce, Rockford, Ill), and exposed to an X-ray film (Kodak, NY). After film development, optical density of immunoblots was quantified using ImageQuant 2.5 (Amersham).

Determination of Tissue Antioxidative Enzyme Activities and Vitamin Concentrations

Coronary artery tissue was homogenized in a potassium phosphate buffer containing 10 mol/L dextroseamine, 0.03% butylated hydroxytoluene, and 2% ethanol, pH 7.4, and equilibrated with nitrogen to reduce auto-oxidation. The homogenate was centrifuged at 1000g for 15 minutes at 4°C; the supernatant was retrieved and centrifuged again at 30 000g for 35 minutes at 4°C. The pellet was separated from the supernatant and both fractions stored at −70°C until the assays were performed as described in previous publications.

Plasma and tissue concentrations of vitamin C and vitamin E were assessed with high-performance liquid chromatography.

Statistical Analysis

Continuous data were expressed as mean±SD in the text and tables, and as mean±SEM in the figures. Comparison of groups was performed using ANOVA, followed by post-hoc tests for parametric and nonparametric distribution. Statistical significance was accepted for P<0.05.

Results

Study Animals

Cholesterol serum concentration was higher in HC than in N and HT (526.16±111.3 versus 81.4±13.5 and 74±14.2 mg/dL; P<0.05 for both comparisons), as was low-density lipoprotein cholesterol serum concentration (395.5±106 versus 38.6±14 and 37.2±6.8 mg/dL; P<0.05 for both comparisons). Mean arterial pressure was higher in HT than in N and HC (141.3±21 versus 107.4±8.9 and 109.4±7.8 mm Hg; P<0.05 for both).

Coronary Artery Antioxidative Enzyme Activities and Vitamin Concentrations

A reduction in tissue concentrations of vitamin E was noted for HC but not for HT, along with a reduction in antioxidant...
enzyme activities with the exception of catalase, which was reduced in both HC and HT (Table 1).

**Histological and Morphometric Analyses**

Regular hematoxylin and eosin staining of coronary artery cross-sections did not show any substantial histological differences between the 3 groups. Morphometric analyses indicated no significant difference among N, HC, and HT regarding intima-to-media (0.08±0.03, 0.11±0.05, and 0.23±0.26), intima-to-lumen (0.09±0.06, 0.10±0.04, and 0.22±0.25), and media-to-lumen ratio or EELLA (1.03±0.32, 0.95±0.29, and 1.01±0.09).

**Microscopic Computed Tomography**

As outlined in Table 2 and illustrated in Figure 1, VV density was highest in HC, with no significant difference between N and HT. Figure 2 shows the VV distribution pattern along the scanned coronary artery segments in the 3 groups, demonstrating that more extensive clustering was apparent in HC with an overall similar distribution pattern in N and HT. Most pronounced in HC and HT, VV count was higher on the epicardial than on the myocardial side of the coronary artery (Figure 2).

**Immunostaining and Immunoblotting**

As shown in Figure 3, collagen III occupied almost twice as much of coronary adventitial area in HT as in N and HC. Along with these changes, significant increase in α-SMC actin immunostaining was observed in HC and further increased in the adventitial layer of coronary arteries from hypertensive animals along with the presence of α-SMC actin-positive fibroblasts (Figure 4). On serial sections, these cells overlapped with vimentin staining. Overall, immunoreactivity for vimentin and TGF-β was significantly higher in HC and HT compared with N (Figure I, available online at http://atvb.ahajournals.org). Immunoreactivity for AngII, ET-1, and VEGF was higher in HC compared with both N and HT (Figures I and II, available online at http://atvb.ahajournals.org). There was no difference in the expression of HIF-1α between HC and HT (Figure II).

**Discussion**

The 2 main findings of the current study are that adventitial remodeling occurs before an alteration in the media-to-lumen area ratio and that it is differentially affected by experimental hypercholesterolemia and hypertension.

**Remodeling of the Vascular Wall**

A number of studies have described the change in vascular structure in atherosclerosis. As initially conceptualized by Glagov, remodeling is classically considered to be positive or centrifugal to preserve lumen dimensions as the atherosclerotic plaque increases in size. In contrast, negative remodeling has been described as a process of reduction of vessel dimension. With regard to both positive and negative remodeling, the focus has been on intima and media, and it has remained questionable whether cardiovascular risk factor exposure leads to structural alteration of the adventitia as well. The current study gives a positive answer to this question and even demonstrates that coronary artery remodeling can be noticed first in the adventitia on exposure to

**TABLE 1. Coronary Artery Antioxidative Enzyme Activities and Vitamin Concentrations**

<table>
<thead>
<tr>
<th></th>
<th>N (n=3)</th>
<th>HC (n=6)</th>
<th>HT (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glut-peroxidase, mU/mg protein</td>
<td>76.33±6.11*</td>
<td>57.67±12.97</td>
<td>68.67±3.51</td>
</tr>
<tr>
<td>Catalase, mU/mg protein</td>
<td>21.67±2.08†</td>
<td>16.17±3.49</td>
<td>14.67±1.53</td>
</tr>
<tr>
<td>CuZn-SOD, mU/mg protein</td>
<td>8.50±0.85</td>
<td>7.08±0.25†</td>
<td>7.43±0.21</td>
</tr>
<tr>
<td>Mn-SOD, mU/mg protein</td>
<td>2.43±0.06</td>
<td>1.92±0.15†</td>
<td>2.17±0.25</td>
</tr>
<tr>
<td>Vitamin E, mg/mg protein</td>
<td>0.66±0.06</td>
<td>0.58±0.04†</td>
<td>0.66±0.03</td>
</tr>
<tr>
<td>Vitamin C, mg/mg protein</td>
<td>1.13±0.26</td>
<td>1.14±0.07</td>
<td>1.18±0.04</td>
</tr>
</tbody>
</table>

*Values are mean±SD.
*P<0.05 vs HC; †P<0.05 vs other 2 groups.

**TABLE 2. Microscopic Computed Tomography Analysis Data**

<table>
<thead>
<tr>
<th></th>
<th>N (n=6)</th>
<th>HC (n=6)</th>
<th>HT (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area, mm²</td>
<td>3.1±1.8</td>
<td>3.8±0.7</td>
<td>3.9±0.1</td>
</tr>
<tr>
<td>Vessel wall area, mm²</td>
<td>3.3±0.2</td>
<td>4.5±0.6</td>
<td>5.3±0.3*</td>
</tr>
<tr>
<td>Vasa vasorum area, mm²</td>
<td>1.8±0.5</td>
<td>3.1±0.4</td>
<td>3.1±1.2</td>
</tr>
<tr>
<td>Vasa vasorum count, n</td>
<td>4.2±1.5</td>
<td>9.3±2.9*</td>
<td>6.8±1.7</td>
</tr>
<tr>
<td>Vasa vasorum density, n/mm²</td>
<td>1.9±0.3</td>
<td>3.4±1.0†</td>
<td>2.0±1.2</td>
</tr>
<tr>
<td>Ratio 2nd/1st order vasa vasorum</td>
<td>3.1±2.4</td>
<td>3.7±1.9</td>
<td>2.5±1.3</td>
</tr>
<tr>
<td>Diameter 1st order vasa vasorum</td>
<td>99.5±32.8</td>
<td>93.3±27.4</td>
<td>81.0±24.5</td>
</tr>
<tr>
<td>Diameter 2nd order vasa vasorum</td>
<td>60.0±13.1</td>
<td>55.5±8.2</td>
<td>77.0±13.9</td>
</tr>
</tbody>
</table>

*Values are mean±SD.
*P<0.05 vs N; †P<0.05 vs other 2 groups.
cardiovascular risk factors such as hypercholesterolemia and hypertension. These findings are reminiscent of previous reports by Shi et al on the effect of balloon injury on the vascular wall.\(^2\) Thus, just like mechanical injury to the vascular wall, functional injury can trigger a response-to-injury response, whose earliest structural alterations seem to become apparent in the adventitia.

**Hypercholesterolemia and Remodeling**

Hypercholesterolemia remains the prototype cardiovascular risk factor for atherosclerotic cardiovascular disease. In animal models of hypercholesterolemia, a sequence of events can be noted, eventually leading to lipid accumulation in the vascular wall. As demonstrated by Heistad et al, increase in adventitial vessel density is an adjunctive phenomenon in hypercholesterolemia, and previous work from our group demonstrated that these changes precede even the development of endothelial dysfunction of the main vessel.\(^9\,17\) The current study extends these previous contributions by demonstrating that adventitial remodeling of the coronary artery wall in hypercholesterolemia occurs at a very early stage of atherogenesis and consists of an increase of extracellular matrix, as reflected by trichrome staining, as well as an increase in cellular components, as reflected by vimentin. In comparison with hypertension, hypercholesterolemia does not seem to be associated with an increase in collagen III content but rather a remarkable increase in VV density in the adventitia of coronary arteries. In line with these findings, Xu et al demonstrated that hypertension superimposed on hypercholesterolemia, but not hypercholesterolemia by itself, is associated with an increase in the expression of collagen I and III within the vascular wall in a murine model.\(^18\) As for the potential underlying mechanism of the more vascular type of remodeling in hypercholesterolemia, it was suggested that VV endothelial dysfunction might impair oxygen supply to the vascular wall, leading to tissue hypoxia with the subsequent expression of pro-angiogenic factors and eventually VV neovascularization.\(^19\,21\) However, previous studies already showed that endothelium-dependent vasodilatation is

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**Figure 1.** Micro-CT images of coronary arteries from normal (N), hypercholesterolemic (HC), and hypertensive (HT) animals. Compared with N and HT, a much more extensive vasa vasorum network can be seen in HC. Reconstruction voxel size: 21 \(\mu\)m.

**Figure 2.** As displayed in the top portion of the figure, the vessel wall can be stratified into quadrants according to myocardial (M) and epicardial (E) orientation. As highlighted by the bar graphs, VV count was significantly \((P<0.05)\) higher on the epicardial side of the vessel wall in hypercholesterolemic (HC) and hypertensive (HT) but not in normal (N) animals. As reflected by the middle panel, the distribution pattern of VV count is much more heterogeneous in HC.

**Figure 3.** Vascular wall fibrosis in coronary arteries from pigs on a normal (N) or hypercholesterolemic diet (HC) or with hypertension (HT) as reflected by (A) trichrome staining and (B) immunoreactivity for collagen III.
impaired to a similar degree in experimental hypercholesterolemia and renovascular hypertension, including epicardial arteries and coronary microvessels, making the “sole hypoxia” theory a less likely explanation for the remodeling differences between them. The current study failed to show a significant difference in the expression of HIF-1α between experimental hypercholesterolemia and renovascular hypertension. Although overlap with hypoxia has to be kept in mind, a potentially significant pathophysiological role of oxidative stress by itself in neovascularization is, nevertheless, supported by the finding that antioxidant vitamin supplementation prevented increase in myocardial microvascular density otherwise seen in hypercholesterolemia. In the current study, antioxidant reserve and scavenger activities were depleted to a much higher degree in hypercholesterolemia than in hypertension, paralleled by an increase in tissue AngII and especially ET-1 levels in hypercholesterolemia. Previous studies already demonstrated that hypercholesterolemia stimulates the tissue renin-angiotensin system, increasing tissue oxidative stress and the expression of ET-1, which can exert additional pro-angiogenic potential. In line with these studies, it may well be that in the presence of similar HIF-1α levels, ET-1 exerted the differential impact on VEGF expression and eventually VV neovascularization in hypercholesterolemia. Thus, one of the earliest changes in hypercholesterolemic coronary artery disease seems to occur in the adventitia and may be characterized as a more vascular type of adventitial remodeling.

### Hypertension and Remodeling

Experimental models of hypertension confirmed alterations in arterial structure relating to the exposure of this cardiovascular risk factor. Kuwahara et al indicated increase in the expression of HIF-1α and VEGF and VV neovascularization of the ascending aorta in rats, made hypertensive by suprarenal aortic constriction. However, Marcus et al did not observe increase in VV in a primate model of renovascular hypertension and, as mentioned, Xu et al found rodent experimental hypertension to be associated with fibrotic rather than vascular changes. This latter finding is in line with study results by Anversa et al, showing that collagen accumulates in the coronary artery wall as early as during the first 2 months of life of spontaneously hypertensive rats, hence being one of the first pathologic effects of hypertension on their vascular wall. Our current results are consistent with these findings, identifying porcine renovascular hypertension to be associated primarily with an increase in adventitial collagen III content, although not overall higher collagen content than experimental hypercholesterolemia. However, as mentioned before by Intengan and Schiffrin, it may be a matter of quality of collagen subtype and cross-linking rather than overall collagen content, which defines the effect on the vascular wall. The overall qualitative difference, found in the current study, is in line with the experimental studies on skin fibroblasts cultivated from patients with essential hypertension, which were found to express collagen III rather than collagen I. In addition, higher density of collagen III but not of collagen I was demonstrated in conduits arteries of rats with genetic hypertension. As a potential source of collagen III production, we found that hypertension but not hypercholesterolemia is associated with the transformation of adventitial fibroblasts into myofibroblasts, which has been linked to the combined action of mechanical tension and TGF-β, particularly TGF-β1, as, for instance, in balloon injury models. In the current study, however, we did not see a difference between hypertensive and hypercholesterolemic pigs with regard to TGF-β tissue immunoreactivity, using an antibody raised against porcine TGF-β1, which may exert greater specificity potential for TGF-β2. Yet, in comparison with pigs on a high-cholesterol diet, coronary arteries of pigs with renovascular hypertension also did not display a higher level of coronary artery expression of AngII, which has been implicated as an important stimulator for TGF-β expression, making the TGF-β–AngII axis a less likely major contributor to this difference in remodeling between these 2 cardiovascular risk factors. It has to be pointed out, though, that this profile was noted in the chronic stage of risk factor exposure, and current findings cannot exclude the possibility that this axis may be stimulated to a higher degree in the early stage of renovas-

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**Figure 4.** Immunostaining for α-SMC actin in coronary arteries from animals on a normal (N) or hypercholesterolemic (HC) diet or with hypertension (HT). At higher magnification (100×, right) bands of α-SMC actin-positive cells can noticed in the adventitia in HT but not in HC or N. The bar graphs on the bottom present the quantitative reflection.
cular hypertension, leading to morphological differences preserved and observed after 12 weeks. Additional alternatives to consider comprise alterations on the side of collagen degradation. In this regard, the balance between matrix metalloproteinases (MMPs) and their inhibitor, tissue inhibitors of matrix metalloproteinases (TIMPs), may be most important, especially MMP-1 and TIMP-1 in cases of collagen III. However, MMP-1 is also a substrate to MMP-2, MMP-3, MMP-7, and MMP-10, and collagen III is substrate to MMP-3, MMP-8, MMP-10, MMP-13, MMP-14, and MMP-16, leading to an array of interactions to the final determination of the level of proteolytic activity. It may be for this reason that previous studies did not provide conclusive evidence that this would be a very prominent mechanism for tissue fibrosis. Thus, hypertensive coronary artery disease is associated with early structural changes of the adventitia with an overall more avascular type of remodeling.

Clinical Implications
The current study indicates that the vascular changes during early exposure to hypercholesterolemia and hypertension are not uniform; hence, these 2 cardiovascular risk factors may influence atherogenesis in different ways, with the potential call for differential therapeutic strategies. The effect of antagonists of the renin-angiotensin system has been shown not only in hypertension but also in hypercholesterolemia. Current study findings support this notion and would even favor additional strategies to target the endogenous endothelin system to ameliorate the vascular response in hypercholesterolemia. Furthermore, current study findings add to the debate on the potential merit of antioxidant strategies, as revived by the promising results of the CART-1 and the interim data of the CART-2 trial. With regard to these clinical implications, it may be pointed out that assessment of EELLA ratios, as commonly performed in the clinical arena, may not capture the entire extent of remodeling, which affects all layers of the vascular wall, including the adventitia at an early stage of the disease process, as outlined by the current findings.

Conclusions
The current study suggests that adventitial remodeling precedes intima and media remodeling of coronary arteries early after exposure to hypercholesterolemia and hypertension with distinct qualitative differences between them.

Acknowledgments
This work was supported by National Institutes of Health grants HL-03621 and HL-63282, the American Heart Association, and the Mayo Foundation. We greatly acknowledge the technical support provided by Catherine Gray (laboratory technologist).

References


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Arterioscler Thromb Vasc Biol. 2005;25:447-453; originally published online December 9, 2004;
doi: 10.1161/01.ATV.0000152606.34120.97

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A. Immunohistochemical staining grade (arbitrary units)

**VIMENTIN**

- **N**
- **HC**
- **HT**

B. Immunohistochemical staining grade (arbitrary units)

**TGF-β**

- **N**
- **HC**
- **HT**

C. Immunohistochemical staining grade (arbitrary units)

**ANG-II**

- **N**
- **HC**
- **HT**
A  Densitometric units

HIF-1 α

NH C H T

0.00 0.05 0.10 0.15 0.20 0.25 0.30

NH C H T

0.00 0.05 0.10 0.15 0.20 0.25 0.30

B  Immunohistological staining grade (arbitrary units)

VEGF

NH C H T

0.00 0.05 0.10 0.15 0.20 0.25 0.30

NH C H T

0.00 0.05 0.10 0.15 0.20 0.25 0.30

C  Immunohistological staining grade (arbitrary units)

ET-1

NH C H T

0.00 1.00 2.00 3.00 4.00 5.00 6.00

NH C H T

0.00 1.00 2.00 3.00 4.00 5.00 6.00

p<0.05 p<0.05

120 kDa