Remodeling of Coronary Vessels During Aging in Purebred Beagles

Robert J. Tomanek, Marian R. Aydelotte, and Ronald J. Torry

We compared six young (1 year) and six senescent (11 years) purebred beagles to determine the effects of aging on the coronary microvasculature. The hearts were perfusion-fixed in vitro, and myocardial specimens were subjected to microscopic image analysis. Absolute left ventricular mass increased by 55% with age, while cardiocyte cross-sectional area increased by 10% and 30% in the midmyocardium and endomyocardium, respectively. Although capillary numerical density was lower in the senescent dogs (16% in midmyocardium and 19% in endomyocardium), volume density was similar in the two groups because capillary diameter increased significantly with age in both regions. Capillary density was reduced by 27% with age in the endomyocardium of the old beagles. Wall/lumen ratios of arterioles of three size classes (≤15, 16–25, and 26–50 μm) were found to be nearly identical for the two age groups. Mean arteriolar diameter increased within the smallest size class in both ventricular regions. In contrast, a twofold or greater increase in wall thickness with age occurred in the left anterior descending coronary artery and its first branch, which was mainly due to expansion of the medial interstitium. The connective tissue fraction of the myocardium was significantly higher in the senescent than in the young in the epimyocardium and midmyocardium but not in the endomyocardium. These data provide evidence for three conclusions regarding aging of coronary vessels in beagles. First, a decline in capillary length density is limited to the endomyocardium. Second, capillary volume density in the aged beagles is maintained by increases in capillary diameter; the latter minimizes a decrement in anatomic intercapillary distance. Third, large coronary arteries undergo substantial medial thickening but no luminal narrowing, and the arterioles are not adversely affected. Accordingly, this species appears to be a good model of coronary vessel aging because senile alterations are minimal. (Circulation Research 1991;69:1068–1074)

Despite evidence that blood vessels are affected by the aging process,1–3 the age-related changes may vary with the specific vascular bed, type of vessel, and species. Although age-associated pathological changes have been reported to occur in coronary arteries in humans and experimental animals, a consistent pattern indicative of physiological aging has not been established. Studies on humans are difficult to interpret because the effects of diet, lifestyle, genetics, and disease cannot be controlled. Most of the experimental studies on the aging of coronary vessels are based on rats, because of their relatively short life span. Earlier descriptive studies suggested that old rats (about 2 years old) develop sclerosis of the intima,4 medial thickening,5 and medial fibrosis.6 A decrease in capillary numerical density7,8 and a decrease in maximal coronary perfusion or coronary reserve9–12 have also been documented in the rat.

The absence of data for coronary vessels of senescent dogs is ostensible because so many studies on the coronary circulation are based on this species. This article, which is the first to document histomorphometric changes that occur over the life span of dogs, is based on data from young-mature (1 year) and senescent (11 years) purebred beagles. The overall goal of the study was to specify the effects of aging on the anatomic characteristics of 1) epicardial arteries, 2) arterioles (precapillary vessels, <50 μm lumen diameter), and 3) capillaries.

Materials and Methods

We compared hearts obtained from six young (1 year old) and six senescent (11 years old) male, purebred beagles. The senescent beagles were purchased at 12 months of age and maintained by the University of Iowa Animal Care Unit in facilities that
allowed free mobility and daily activity. Male beagles are considered adult at age 7–8 months. At the time of study the dogs were anesthetized with thiopental sodium (30 mg/kg i.v.) and α-chloralose (80 mg/kg i.v.). After the completion of studies on diastolic left ventricular filling and arterial baroreceptors, the heart was arrested in diastole by an intraventricular injection of procaine (200 mg) and was rapidly excised; a large-bore catheter was positioned and secured in the ascending aorta. Two liters of Locke’s solution was perfused via a peristaltic pump through the coronary vasculature and was immediately followed by 4 l of a glutaraldehyde-parafomaldehyde fixative solution (1.5% glutaraldehyde, 0.2% paraformaldehyde, 0.1 M CaCl₂, 0.1 M cacodylate, pH 7.4). The perfusions were performed at a driving pressure of 120 mm Hg. The heart was immersed in the fixative overnight and then placed in 0.1 M cacodylate buffer containing 3% sucrose. The left anterior descending coronary artery and its first branch were dissected near their points of origin. Blocks of tissue were excised from three layers of the left ventricular free wall (epimycocardium, midmyocardium, and endomyocardium) with careful reference to the plane of orientation of cardiocytes. Accordingly, the tissues could be subsequently sectioned at a right angle to the long axis of the cells. The epimycocardial specimens were used for general histology and morphometry of connective tissue only, whereas the specimens from the other regions were used for all analyses. After dehydration, myocardiad tissue specimens were embedded in JB-4 (methyl methacrylate) and the two coronary arteries were embedded in Spurr’s plastic. Tissue sections (1.5 μm for JB-4 and 1.0 μm for Spurr’s) were cut from the blocks. Sections from the JB-4 blocks were placed on glass slides and stained with 1) hematoxylin and eosin or 2) modified periodic acid methenamine silver procedure with light green as a counterstain. The epicardial artery slides were stained with Richardson’s solution.

Histomorphometric Analyses

All morphometric analyses were performed with a BioQuant image analysis system interfaced to a Nikon Labophot microscope. Capillary profile long and short axes were used to estimate capillary volume density (Vc) as follows:

\[ V_c = \pi (r_1 r_2) (N_A) \]

where \( r_1 \) and \( r_2 \) are the radii of the long and short axes and \( N_A \) is capillary numerical density. The long and short axis ratio (a/b) was used to compute length density (Lc) according to the formula

\[ L_c = a/b (N_A) \]

Because capillaries may deviate from a perfect cylindrical shape, \( L_c \) may be slightly overestimated by this procedure. However, intergroup comparisons should be valid. Anatomic intercapillary distance (ICD) (i.e., the mean distance between peripheries of adjacent capillaries) was calculated using the formula of Bassingthwaite et al:

\[ ICD = \sqrt{\frac{2A}{N_A \sqrt{3} - \bar{X}_d}} \]

where A is the tissue area and \( \bar{X}_d \) is the mean capillary diameter (shortest axis) of the specimen. These capillary data are based on measurements from approximately 300 capillaries/specimen.

The shortest luminal axis of an artery or arteriole was recorded as the vessel’s diameter, while wall thickness (media and intima) was measured at the two points where the line defining the lumen diameter contacted the vessel’s wall. The mean of the two wall values was used. Arteriolar measurements were made at a magnification of ×2,100 and were obtained from 75–100 arterioles/specimen. Diameters of arterioles and their wall thickness were determined at magnifications of ×68 and ×1,380, respectively. Arterioles were divided into three size (lumen diameter) classes (≤15, 16–25, and 26–50 μm) for analysis. Of the total number of arterioles evaluated, 53%, 29%, and 18% fell into the three size classes, respectively. Cross-sectional fields of tissue specimens were systematically scanned, and all arterioles within a given field were measured.

Cardiocyte boundaries were clearly demarcated in slides stained with silver. Cross-sectional myocyte profiles containing a nuclear profile were measured with the BioQuant image analysis system. These slides were also used for connective tissue volume density, a parameter based on the contrast between the silver-stained extracellular compartment and other tissue components.

Statistical Analysis

All data were based on analysis of variance and the least-squares mean method. Student’s t test for unpaired data (\( p<0.05 \)) was used to denote statistical significance.

Results

Arterial Pressure and Heart Rate

Physiological data for five of the six young dogs used in our study and for 11 old dogs, which included the six used in our study, have been recently published. Mean arterial pressure was found to increase from 110±7 mm Hg in the young to 127±7 mm Hg in the old. Resting heart rate also rose with age from 78±9 to 111±8 beats/min.

Body Weight, Left Ventricular Weight, and Cardiocyte Size

Table 1 shows body weight, left ventricular weight, and cardiocyte size for young and old dogs. The old dogs were 59% heavier than their young counterparts. While left ventricular mass increased 55%, right ventricular mass (young, 20.0±2.6 g; senescent, 24.6±2.6 g) increased only 23% with age. Left ventricular weight/

body weight ratios were similar for the two groups. Differences in cardiomyocyte cross-sectional area between the two age groups are most notable in the endomyocardium, where a 30% increase with age occurred.

Coronary Arteries and Arterioles

Data for the left anterior descending coronary artery and its first branch are listed in Table 2. Despite the large increase in left ventricular mass with age, lumen diameters of these two epicardial arteries did not increase significantly with age; however, wall thickness and wall/lumen ratio increased by a factor of approximately two with age. We found that the nonmuscle components (interstitium) of the tunica media were approximately twofold higher in the senescent compared with the young beagles. When the muscle and interstitial compartments of the media are expressed as absolute values (per 1-μm length of vessel), it can be shown that both components expanded during aging. Interstitial volume increased sevenfold and 10-fold in the left anterior descending coronary artery and its branch, respectively, whereas smooth muscle volume expanded by only 94% and 54%, respectively, in the two vessels. Thus, the increase in medial thickness was mainly the consequence of interstitial growth. However, a significant, albeit less pronounced, expansion of muscle also occurred.

Figure 1 illustrates the mean luminal diameters of small arteries in three size classes in two transmural locations (midmyocardium and endomyocardium). In both of these regions mean luminal diameters of the smallest arterioles (≤15 μm) from old beagles were larger than those from the young group. Mean diameters in the 16–25 and 26–50 μm classes were nearly identical. Arteriolar wall/lumen ratios were similar for the two age groups within each size category and in both myocardial regions (Figure 2).

Capillaries

Capillary numerical density, diameter, and anatomic intercapillary distance are shown in Table 3. Compared with the young group, capillary numerical density was significantly lower in the senescent beagles in both midmyocardium and endomyocardium, whereas capillary diameter was significantly larger in the endomyocardium of the old beagles. Mean anatomic intercapillary distance did not differ significantly between the two groups. This parameter was similar for both age groups within a given ventricular region because of the offsetting effects of capillary diameter and numerical density; that is, the beagles with lower numerical densities tended to have larger diameters. Capillary volume density tended to be higher in the old compared with the young beagles (Figure 3); however, the differences did not attain statistical significance. Capillary length density in the endomyocardial region was 27% lower in the old than in the young beagles (Figure 4). In contrast, length density was not significantly affected by age in the midmyocardium.

Table 1. Body Weight, Left Ventricular Weight, and Cardiocyte Size

<table>
<thead>
<tr>
<th></th>
<th>Young (n=6)</th>
<th>Old (n=6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>8.6±0.7</td>
<td>13.7±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>53.6±6.3</td>
<td>83.0±6.3</td>
<td>0.005</td>
</tr>
<tr>
<td>LV/BW (g/kg)</td>
<td>6.25±0.64</td>
<td>6.19±0.64</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiocyte cross-sectional area (μm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>230±17</td>
<td>253±17</td>
<td>NS</td>
</tr>
<tr>
<td>Endomyocardium</td>
<td>192±14</td>
<td>250±14</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BW, body weight; LV, left ventricular.

Table 2. Morphometric Data for Epicardial Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>Lumen diameter (μm)</th>
<th>Medial thickness (μm)</th>
<th>Wall/lumen ratio</th>
<th>Volume</th>
<th>Absolute volume (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Interstitium</td>
<td>% Myocytes</td>
</tr>
<tr>
<td>LAD</td>
<td></td>
<td></td>
<td></td>
<td>Interstitium</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Young</td>
<td>1,418±136</td>
<td>41±7</td>
<td>0.029±0.011</td>
<td>22.6±0.6</td>
<td>77.5±3.3</td>
</tr>
<tr>
<td>Senescent</td>
<td>1,618±152</td>
<td>105±7</td>
<td>0.072±0.012</td>
<td>51.5±4.4</td>
<td>48.3±5.1</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>0.001</td>
<td>0.0005</td>
</tr>
<tr>
<td>LAD branch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>849±89</td>
<td>31±6</td>
<td>0.040±0.012</td>
<td>12.8±2.5</td>
<td>87.2±2.3</td>
</tr>
<tr>
<td>Senescent</td>
<td>945±100</td>
<td>67±7</td>
<td>0.076±0.013</td>
<td>46.5±3.0</td>
<td>53.5±3.4</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>0.001</td>
<td>0.025</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Lumen diameter, medial thickness, and wall/lumen ratio are based on vessels from six young and five senescent dogs. Myocyte and interstitium data derived from the tunica media are from six young and four senescent dogs. Absolute volumes of interstitium and myocytes are based on 1-μm vessel length. Standard errors for absolute volumes of interstitium were derived from individual group variances since the variance differed markedly owing to the large differences between means. LAD, left anterior descending coronary artery.
Regional Myocardial Connective Tissue Volume Density

Using sections stained with modified periodic acid silver methenamine, we determined connective tissue volume density in three myocardial regions (epimycocardium, midmyocardium, and endomycocardium). As illustrated in Figure 5, the volume percent of connective tissue in the epimycocardium and midmyocardium was about 2.5-3.0% in the young group but nearly 4% in the old beagles. Both groups had virtually identical values for the endocardium (5.1%). This region also showed the greatest animal-to-animal variability. Thus, in beagles, myocardial connective tissue increases with age in the epimycocardial and midmyocardial regions but still occupies less volume than that in the endomycocardium. It is emphasized that our measurements were derived from myocardial specimens and therefore did not include the epicardial or endocardial layers or fields with large vessels.

Cardiocyte volume density can be estimated by subtracting mean connective tissue and capillary volume densities from 100%. Accordingly, cardiocyte volume densities for the young beagles are 86% and 85% in the midmyocardial and endomycocardial regions, respectively. In the senescent group the respective values are 84% and 83%.

Qualitative Histology

As illustrated in Figure 6 and quantified in Table 2, the extracellular compartment of the tunica media is considerably larger in the specimens from the old dogs. We also noted that the smooth muscle cells in the old beagles were not as uniformly oriented as those of their young counterparts (Figure 6). No consistent age-related differences in the intima were noted. Therefore, wall thickness measurements were not affected by variability in the intima, which constituted a negligible portion of the vascular wall. However, on rare occasions we observed a focal wall thickening in arterioles from old beagles, which gave rise to a slight protrusion into the vessel’s lumen. We emphasize that such rare observations describe a focal wall protrusion that did not provide any evidence of vascular occlusion. Other than these changes, the histological appearance of the tissue sections from the two age groups revealed few differences. Cardiocytes were similar in the two groups and failed to show any trends of degenerative changes in the old animals. Perivascular fibrosis was not marked in the old beagles, nor did we observe any evidence of replacement fibrosis. The endomycocardial region, where such changes might be expected, did not appear altered, an observation that is consistent with the quantitative data for connective tissue presented above.

Figure 1. Mean arteriolar lumen diameters (±SEM) for three size classes of arterioles (according to lumen diameters: 0–15, 16–25, 26–50 μm).

Figure 2. Arteriolar wall:lumen ratios (mean±SEM) of three size classes of arterioles. None of the intergroup differences, for any size class, are statistically significant.
TABLE 3. Capillary Numerical Density and Diameter and Anatomic Intercapillary Distance

<table>
<thead>
<tr>
<th></th>
<th>Young (n=6)</th>
<th>Old (n=6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerical density (No./mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>4.580±0.219</td>
<td>3.869±0.219</td>
<td>0.03</td>
</tr>
<tr>
<td>Endomyocardium</td>
<td>4.582±0.204</td>
<td>3.729±0.204</td>
<td>0.009</td>
</tr>
<tr>
<td>Diameter (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>4.88±0.30</td>
<td>5.35±0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Endomyocardium</td>
<td>4.37±0.17</td>
<td>5.76±0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intercapillary distance (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>11.04±0.50</td>
<td>12.03±0.50</td>
<td>NS</td>
</tr>
<tr>
<td>Endomyocardium</td>
<td>13.48±0.73</td>
<td>14.93±0.73</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Discussion

Few studies have addressed the role of aging on the coronary circulation. This study is the first to provide data for the effects of aging on coronary vessels in a canine model. By using purebred beagles, we met important genetic and environmental requirements necessary in experimental aging studies. Three of our findings support the thesis that vascular remodeling occurs during the aging process. First, wall thickness and wall/lumen ratio of epicardial arteries increased with age, but lumen diameter was unchanged. Second, although wall thickness and wall/lumen ratio of arterioles did not change during aging, mean luminal diameter of the smallest class of arterioles (≤15 μm) increased. Finally, although capillary diameter increased and numerical density decreased, volume density and anatomic intercapillary distance remained relatively constant. However, length density, an important measure of capillarity, decreased by 27% in the endomyocardium. Thus, all three classes of coronary vessels (arteries, the smallest arterioles, and capillaries) undergo changes over the life span. However, many of the changes may be adaptive rather than degenerative.

Although an increase in medial thickness of coronary epicardial arteries with aging may not be surprising, the more than twofold increase in wall thickness and the negligible increase in internal diameter in the present study are remarkable. The latter is consistent with observations in humans that the coronary arterial lumens do not enlarge with age.18 Our data also show that enhancement in the extracellular compartment of the tunica media contributed substantially to wall thickening. Unlike observations for humans19 and rats,20 however, arterial wall thickness was not increased with age in beagles.

Arterial blood pressure is one important factor that may influence the structure of arteries and arterioles. Several studies have documented increased wall/lumen ratios in coronary arterioles of rats with spontaneous,21-23 deoxycorticosterone acetate–salt,24 or renal20,25 hypertension. We recently demonstrated that the wall/lumen ratio of small (<50 μm luminal diameter) coronary arterioles of rats increases between mid-life and senescence.20 Such data at least suggest a similarity between changes in coronary arterioles during aging and hypertension. Evidence that age-associated intimal thickening of
the aorta can be prevented by chronically lowered arterial pressure in rats further implicates the role of intravascular pressure as a factor in vascular alterations over time.\textsuperscript{26} Indeed, our study demonstrates that the old dogs had higher mean arterial pressures than their young counterparts, and another study reported an elevated systemic vascular resistance in old compared with young adult beagles.\textsuperscript{27} However, our earlier work with mongrel dogs shows that hypertension (one-kidney, one-clip) does not produce the same changes in coronary vessels as those found in rats. Although the media of epicardial arteries were marginally thickened because of a significant enhancement of connective tissue after 6 weeks of hypertension, a finding consistent with hypertensive rats,\textsuperscript{28} luminal diameter increased.\textsuperscript{29} In contrast to the studies on rats, wall/lumen ratios of arterioles and arteries were not affected by either 6 weeks\textsuperscript{29} or 7 months\textsuperscript{30} of hypertension. Considering this evidence it appears that the response of arterioles to aging, as well as hypertension, is species dependent. The age-related decrease in capillary numerical and length density in beagles is consistent with previous data for rats.\textsuperscript{7,8} Yet, our findings indicate that capillary volume density and anatomic intercapillary distance are not compromised by age because capillary diameter is larger in senescent than in young dogs. Thus, in the senescent heart the mean distance between the edges of adjacent capillaries is not significantly increased. In the endomyocardium the 27\% decrement in capillary length density is accompanied by a 32\% increase in diameter. The consequences of this remodeling on tissue oxygenation are not evident, because concepts of capillarity and O\textsubscript{2} diffusion distances are open to various interpretations.\textsuperscript{31}

Perhaps the most surprising finding is the relatively mild increase in cardiocyte cross-sectional area and the lack of evidence of degenerative changes in these cells. These data, along with the fact that heart mass increased substantially with age, indicate that cell loss was not likely, unless it was compensated by cardiocyte proliferation. Cell degeneration and loss with age in rats have been reported in several studies.\textsuperscript{32-34} Although cell length was not measured in this study, we expect that it increased consistently with increases in ventricular volume. If cell length increased in proportion to the 30\% enhancement of cross-sectional area, then one could easily account for the 35\% increase in left ventricular mass. However, cardiocyte cross-sectional area was only 10\% (nonsignificant) higher in the midmyocardium. It is possible that myocyte growth was greater in ventricular regions not evaluated in this study. We cannot, however, rule out the possibility of cardiocyte proliferation, which has been recently suggested by Anversa and colleagues.\textsuperscript{34} Although we found connective tissue volume density to be higher in two layers of the left ventricle in the senescent beagles compared with those from the young group, we did not observe any consistent evidence of connective tissue foci that would suggest replacement fibrosis. The latter fits with our failure to find evidence of cell degeneration and loss.

Taking into account all of the findings of this study, we do not find any definitive anatomic evidence that might underlie perfusion abnormalities. Although such abnormalities have not, to our knowledge, been demonstrated in aged beagles, maximal coronary perfusion of isolated nonworking hearts has been found to be compromised in senescent rats.\textsuperscript{9} However, more recent work suggests that this decrement occurs by the first year\textsuperscript{11} and therefore is not a consequence of senescence. It has been shown that large and medium-sized coronary arteries of aged beagles show suppressed relaxations or may even undergo slight contraction in response to norepinephrine and epinephrine.\textsuperscript{35} Data from that study also suggest that \(\beta\)-adrenoceptor function decreases with age, while \(\alpha\)-adrenoceptor function increases with age. In contrast, small coronary arteries of young and senescent beagles had similar magnitudes of relaxation for a given dose of catecholamine.

We recognize that the enhanced nonmuscle compartment of the tunica media documented in our study could contribute to a blunted relaxation of coronary arteries. This postulate is consistent with recent evidence that cerebral arterioles in old rats undergo a reduction in distensibility associated with a decrease in the relative proportion of the distensible elements elastin and smooth muscle.\textsuperscript{36} Whether a compromised relaxation in large coronary arteries would limit myocardial perfusion is not established nor have perfusion deficits in aged dogs been documented. The finding that lumen diameter is not reduced in either arteries or

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Light micrographs of left arteriolar descending coronary arteries; 1-\(\mu\)m-thick cross-section, stained with Richardson's stain. A comparison of a left anterior descending coronary artery from a young (panel A) and old (panel B) beagle illustrates the thicker media with increased connective tissue characteristic of the old beagles. Arrowheads demarcate the intima-media. Bar, 100 \(\mu\)m.}
\end{figure}
arthroles fixed in the relaxed state suggests that maximal flow in these vessels is not limited by increased wall thickness in the aged beagles. Although intercapillary distances are not significantly increased, capillary remodeling occurs with age. Nevertheless, length density is decreased in the endomyocardium and could be a significant factor in O\textsubscript{2} supply during high O\textsubscript{2} demand.

Acknowledgments

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References


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