Morphometry of Human Coronary Capillaries During Normal Growth and the Effect of Age in Left Ventricular Pressure-Overload Hypertrophy

Karel Rakusan, MD, PhD; Michael F. Flanagan, MD; Tal Geva, MD; James Southern, MD, PhD; and Richard Van Praagh, MD

Background. In adults, acquired pressure-overload left ventricular hypertrophy can result in myocardial ischemia, which may be due to part in insufficient capillary growth during development of hypertension. The coronary microvascular response to congenital pressure-overload hypertrophy in children has not been previously characterized.

Methods and Results. Average capillary density and heterogeneity of capillary spacing were measured in 63 postmortem human hearts with left ventricular hypertrophy and control hearts without heart disease. Pathology specimens were chosen that had left ventricular hypertrophy caused by 1) congenital isolated aortic valve stenosis in infants < 1 year old at death, children 9–14 years old, and adults 15–30 years old; 2) congenital isolated coarctation of the aorta in adults 15–39 years old; and 3) acquired aortic stenosis in adults 51–86 years old. Major findings of the study were: 1) Human left ventricular capillary density and heterogeneity of capillary spacing are similar to other mammalian species. 2) Capillary density is higher in infants (3,315 ± 85 capillaries per square millimeter), decreases with increasing heart weight during normal growth in early childhood (children, 2,388 ± 75 capillaries per square millimeter, p < 0.05), and thereafter remains relatively constant. 3) Capillary density with left ventricular hypertrophy is dependent on the age of onset. Congenital aortic stenosis and coarctation are characterized by an increase in capillary supply proportional to myocyte volume, maintaining capillary density similar to control hearts. Adults with acquired aortic stenosis have decreased capillary density (1,671 ± 66 capillaries per square millimeter, p < 0.01 versus control).

Conclusions. Pressure-overload left ventricular hypertrophy in children demonstrates proportional capillary angiogenesis, whereas in adults, hypertrophy appears to be associated with failure of compensatory angiogenesis. (Circulation 1992;86:38–46)

KEY WORDS • myocardium • hypertrophy • coronary circulation • capillaries

Left ventricular hypertrophy as a result of pressure overload is a common clinical disorder associated with significant risk for sudden cardiac death. In younger children, the incidence of sudden death with pressure overload caused by aortic stenosis appears to be substantially less, and it appears to increase with age during childhood and young adulthood.1 Aortic valve stenosis is a leading cause of sudden death from cardiovascular disease not treated surgically2 among adolescents. The etiology of sudden death in patients with left ventricular pressure-overload hypertrophy is complex and may involve multiple causes, including an age-dependent adverse effect of left ventricular hypertrophy.3–5 The mechanism by which left ventricular hypertrophy may be associated with sudden death is unclear and probably multifactorial,6 but myocardial ischemia appears to be an important component. Studies in adult experimental animals7 and adult human patients with aortic stenosis8 have demonstrated a limitation of maximum myocardial perfusion and coronary flow reserve that could lead to myocardial ischemia at times of increased myocardial oxygen consumption.8–10

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This is also supported by the finding that many patients with pressure-overload left ventricular hypertrophy have symptoms, ECG signs, and pathological findings consistent with subendomyocardial ischemia.1 Ischemia11 and ischemia-induced subendomyocardial fibrosis12,13 have also been associated with ventricular dysrhythmias that may be directly causative of sudden death in patients with pressure-overload left ventricular hypertrophy.1,5

Coronary capillaries play a crucial role in the transport of oxygen and nutrients to the myocardium. Al-
though the overall control of coronary blood flow is a function of mainly the small arteries and arterioles, capillary density and distribution in space greatly influence the exchange processes between blood and tissue.14-16 These transport mechanisms are critical for normal cardiac function, and changes associated with cardiac hypertrophy in either one might contribute to the impaired function of the hypertrophic heart.10-17

Morphometric studies in animal models have suggested that inadequate growth of the coronary microvascular bed is one factor limiting myocardial perfusion in mature hearts with pressure-overload hypertrophy.8,10,14-17,22 Studies manipulating coronary microvascular growth during the development of left ventricular hypertrophy in the young suggest that angiogenesis is an important factor in maintaining coronary capillary density22-24 and coronary flow capacity per unit of myocardium.22 Coronary capillary and arteriolar density are diminished in the mature left ventricle with pressure-overload hypertrophy,18,19,25-28 presumably as a result of an increase in myocardial mass without parallel growth of the microvascular bed. Pressure-overload hypertrophy in young growing animals (in contrast to adults) is accompanied by proportionate coronary capillary growth17,21 and normal capillary density and coronary flow capacity.21,29,30 The available data, although few, seem to support the hypothesis that age-dependent coronary vascular growth with pressure overload occurs in humans.28,31-33 To the best of our knowledge, no systematic studies have been done on the effect of age on coronary capillaries in humans during normal growth and with clearly defined isolated pressure-overload hypertrophy.

In the present study, the capillary density and heterogeneity of capillary spacing in human hearts were examined to determine whether the age of the individual during the development of hypertrophy influenced the coronary angiogenic response to pressure-overload hypertrophy. Well-documented cases of isolated pressure-overload left ventricular hypertrophy from several collections of pathology specimens were used to assemble adequate numbers of normal and pathological hearts from subjects of various ages.

Two groups of hearts with aortic stenosis were studied and compared with age-matched controls. The first comprised hearts from infants, children, and young adults with congenital aortic stenosis and coarctation. The second group comprised individuals with aortic stenosis in whom the pressure overload was acquired during adulthood. The results demonstrate the effect of age on capillary density in the normal and hypertrophied human heart.

### Methods

**Animal Studies**

Preliminary studies were performed in rats to determine whether capillary density is significantly influenced by immersion fixation in 10% formalin, as done in clinical pathology specimens, in comparison with perfusion fixation, which has been customary in most capillary morphometric studies in animals.9,17,19 The effect of fixation techniques was studied in 24 adult male Sprague-Dawley rats (Charles River, Montreal) equally divided into three experimental groups: 1) hearts fixed by perfusion with glutaraldehyde; 2) hearts fixed by immersion into glutaraldehyde; and 3) hearts fixed by immersion into formalin.

Histological techniques have been described previously.19 The rats were anesthetized with intraperitoneal sodium pentobarbital, and their hearts were stopped by infusion of potassium chloride into the jugular vein. Hearts from the first experimental group were then fixed in situ by infusion of 1.5% glutaraldehyde buffered to pH 7.4 with 1.5% phosphate buffer at a pressure of 80–90 mm Hg for 20 minutes. Hearts from the remaining rats were fixed by immersion either in the same glutaraldehyde solution or in 10% neutral buffered formalin. After fixation and subsequent dehydration in alcohol, the samples were embedded in historesin.

Capillaries were evaluated from 1-μm-thick sections stained by Avallone's modification of Jones' silver methenamine method for staining basement membranes.

Photomicrographs with an area of 23,500 μm² were obtained of tissue cross sections from the midmyocardium and endomyocardium of the left ventricular free wall. The average density of capillaries and myocytes was measured in both regions on five to seven photomicrographs from each region as previously described.23 The group identity of the photomicrographs was unknown to the examiner during the measurements.

In our previous experience,20 fixation by perfusion with glutaraldehyde yielded optimal conditions for subsequent staining and evaluation of capillary density. Therefore, values of capillary and myocyte density obtained by this method were used as standards for comparisons with those obtained by immersion and by fixation. A disadvantage of the perfusion-fixation method with glutaraldehyde was a significant increase in cardiac weight caused by an increased water content. The effects of various types of fixation on cardiac morphometric parameters studied in adult Sprague-Dawley rats are summarized in Table 1. Hearts fixed by immersion in glutaraldehyde or formalin did not change weight as a result of fixation. Morphometric parameters, including capillary and myocyte density, were very similar to those obtained

### Table 1. Effect of Fixation on Cardiac Morphological Parameters

<table>
<thead>
<tr>
<th>Group 1: Hearts fixed by perfusion with glutaraldehyde</th>
<th>Rats (n)</th>
<th>Body weight (g)</th>
<th>Heart weight (mg)</th>
<th>Myocytes (No./mm²)</th>
<th>Capillaries (No./mm²)</th>
<th>Myocytes/capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>193±2</td>
<td>882±25</td>
<td>3,117±150</td>
<td>3,182±193</td>
<td>0.98±0.01</td>
<td></td>
</tr>
<tr>
<td>Group 2: Hearts fixed by immersion in glutaraldehyde</td>
<td>8</td>
<td>209±3*</td>
<td>658±17*</td>
<td>3,002±117</td>
<td>3,040±79</td>
<td>0.99±0.02</td>
</tr>
<tr>
<td>Difference from group 1 (%)</td>
<td>+8</td>
<td>-25</td>
<td>-4</td>
<td>+1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3: Hearts fixed by immersion in formalin</td>
<td>8</td>
<td>215±3*</td>
<td>672±20*</td>
<td>3,053±111</td>
<td>3,043±86</td>
<td>1.00±0.02</td>
</tr>
<tr>
<td>Difference from group 1 (%)</td>
<td>+11</td>
<td>-24</td>
<td>-2</td>
<td>+2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM.  
*Difference significant at p<0.01.
TABLE 2. Groups of Human Hearts With Aortic Stenosis Studied

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>Control hearts</td>
</tr>
<tr>
<td></td>
<td>Congenital aortic stenosis</td>
</tr>
<tr>
<td>Children</td>
<td>Control hearts</td>
</tr>
<tr>
<td></td>
<td>Congenital aortic stenosis</td>
</tr>
</tbody>
</table>

from hearts fixed by perfusion with glutaraldehyde. Only a small (4%), nonsignificant (compared with glutaraldehyde perfusion) decrease in capillary density was detected in hearts fixed by immersion in formalin or glutaraldehyde. There was no increase in the scatter of measured capillary density in hearts fixed by immersion in formalin.

**Human Subject Material**

Morphometric analysis was performed on 63 human hearts. Hearts with severe isolated aortic stenosis were identified in the age ranges specified from the heart collections at the Cardiac Registry at Children’s Hospital and the pathology department collections at Massachusetts General Hospital and Brigham and Women’s Hospital in Boston. Hearts with additional coexisting congenital or acquired heart disease were excluded. Age-matched control hearts were selected from individuals who died with no apparent left ventricular disease. Patients with chronic debilitating disease and low body weight for age were excluded. Some adult controls had isolated mitral stenosis without clinical or pathological evidence of mitral regurgitation, aortic valve disease, or coronary disease.

Two primary groups of hearts with aortic stenosis were studied (Table 2). The first group consisted of hearts with isolated congenital aortic valve stenosis in three subgroups according to age: infants <1 year old, children 9–14 years old, and adults 15–30 years old. These patients had severely malformed unicommissural or bicommissural aortic valves and no other cardiac anomaly. A fourth additional subgroup of young adults 15–30 years old with isolated congenital coarctation of the aorta was examined to investigate the possible additional effect of coronary hypertension seen in this group in contrast to the age-matched adult group with isolated aortic stenosis. The second main group consisted of adults with acquired calcific or rheumatic stenosis of a trileaflet or bileaflet aortic valve. Not one of these patients was known to have had cardiac disease or murmurs in childhood or to have had significant regurgitant lesions of the mitral valve.

All human hearts had been fixed by immersion in formalin before sections were cut off. Transmural samples parallel to the long axis of the heart were taken from the lateral left ventricular free wall, starting between the heads of the papillary muscles and extending 10–15 mm toward the apex. Samples of formalin-fixed human hearts were dehydrated in alcohol, embedded, sectioned, and stained in a fashion similar to the rat myocardium.

**Morphometric Analysis of Human Coronary Capillaries**

Capillary density was measured in tissue cross-sections on photomicrographs as described above. The results from the midmyocardium and endomyocardium were pooled. The average radius of the Krogh tissue cylinder was calculated from the capillary density. This radius, calculated as the mean distance halfway between two capillaries in cross section, defines the radius of the average tissue cylinder perfused by a single capillary.

Tissue oxygenation is influenced not only by the mean values of the Krogh cylinder but also by the variability of the capillary spacing. For instance, a large heterogeneity of capillary spacing could result in a portion of the myocardium being at a significantly greater distance from the closest capillary. In the human hearts, in addition to mean capillary density, the heterogeneity of capillary spacing was evaluated by the method of capillary domains. An image analyzer (Bioquant IV, Leitz, FRG) was used to locate the position of all capillaries in a tissue cross section and to calculate equidistant border lines between each capillary. The polygonal region around each capillary was defined as the domain area, and the equivalent radius of the Krogh tissue cylinder with the same area was calculated by an on-line computer (Figure 1). The frequency distribution of these radii was approximately log-normal. The standard deviation of the log radii (SD log) was used as a heterogeneity index. A larger SD log indicates a more pronounced heterogeneity of capillary spacing. Because the hearts were not fixed by perfusion at known pressure immediately after death, capillary luminal area was not measured.

The number of cardiac myocytes, and therefore capillary myocyte ratio, was not determined because the myocytes were not clearly outlined in some areas of some slides, and the accuracy of the overall count was therefore reduced. Instead, the relative cross-sectional area occupied by the myocytes was measured by the point-counting method using the same image analyzer, with the results expressed as the percentage of the tissue area occupied by myocytes. Because the capillary luminal area could not be accurately measured, the relative area of the interstitium occupied by capillaries versus fibrosis was not assessed. The examiner was blinded to the group identity of the photomicrographs during all morphometric analyses.

**Statistical Analysis**

Statistical evaluation to test for the effect of age on the response to hypertrophy was performed with a two-way ANOVA. A one-way ANOVA with a subsequent Scheffe test was used to test for the significance of differences between group mean values in rat hearts and in adult human hearts. A value of \( p < 0.05 \) was considered significant.

**Results**

**Developmental Studies: Normal Subjects**

Capillary density and its related indexes (capillary domain area and its radius) were significantly influ-
enced by age and cardiac growth: With increasing age and fourfold growth of the cardiac weight between infancy and childhood (9–14 years), the density of coronary capillaries decreased by 28%, with a corresponding increase in the area and radius of capillary domain (Table 3). During later years of maturation, between the ages of 9 and 14 years and 15 and 30 years, the relative increase in heart weight was much less (56%), and the decrease in capillary density was small (6%). Figures 2 and 3 define changes in capillary density and capillary domain area with increasing cardiac weight (see below).

The proportion of area occupied by myocytes was significantly higher in infant hearts than in older age groups (87% versus 82%, respectively). Correspondingly, the proportion of myocardium occupied by nonvascular interstitial components probably rose between infancy and childhood. There were no apparent differences in the relative area occupied by myocytes between children 9–11 years old and young adults.

### Congenital Aortic Stenosis

Data from hearts with congenital aortic stenosis are summarized in Table 3. The three developmental groups, designated as infants, children, and adults, were equally matched with controls in age, number, body weight, and body surface area. The total heart weight,

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (kg)</th>
<th>Body surface area (m²)</th>
<th>Heart weight (g)</th>
<th>Capillary density (No./mm²)</th>
<th>Capillary domain area (μm²)</th>
<th>Capillary domain radius (μm)</th>
<th>SD log domain radius ×1,000</th>
<th>Myocyte fractional area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control hearts (n=8)</td>
<td>5.6±0.8*</td>
<td>0.27±0.04*</td>
<td>40±6†</td>
<td>3,315±85*</td>
<td>308±9*</td>
<td>9.8±0.1*</td>
<td>64±1</td>
<td>86.8±0.5*</td>
</tr>
<tr>
<td>Aortic stenosis (n=6)</td>
<td>5.1±0.9</td>
<td>0.26±0.04</td>
<td>114±16</td>
<td>3,472±190</td>
<td>292±13</td>
<td>9.6±0.2</td>
<td>70±6</td>
<td>84.5±1.5</td>
</tr>
<tr>
<td>Children (9–14 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control hearts (n=8)</td>
<td>37.5±2.3*</td>
<td>1.20±0.05*</td>
<td>178±8†</td>
<td>2,388±75*</td>
<td>421±13*</td>
<td>11.6±0.2*</td>
<td>59±3</td>
<td>82.4±0.8</td>
</tr>
<tr>
<td>Aortic stenosis (n=7)</td>
<td>39.1±7.2</td>
<td>1.28±0.14</td>
<td>457±64</td>
<td>2,575±167</td>
<td>399±27</td>
<td>11.2±0.4</td>
<td>63±2</td>
<td>81.4±1.9</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control hearts (n=8)</td>
<td>58.5±2.6*</td>
<td>1.61±0.04†</td>
<td>255±24†</td>
<td>2,249±85*</td>
<td>449±17*</td>
<td>12.0±0.2*</td>
<td>59±1</td>
<td>82.1±0.9</td>
</tr>
<tr>
<td>Aortic stenosis (n=7)</td>
<td>70.2±3.1</td>
<td>1.78±0.07</td>
<td>780±48</td>
<td>2,102±103</td>
<td>482±22</td>
<td>12.4±0.3</td>
<td>73±14</td>
<td>82.4±1.3</td>
</tr>
</tbody>
</table>

SD log, standard deviation of the log radii. Values are mean±SEM.
*p<0.01 compared with following age group.
†p<0.01, control heart compared with aortic stenosis.
CAPILLARY DENSITY IN HUMAN HEART DURING NORMAL AND PATHOLOGICAL GROWTH

![Graph showing changes in capillary density with increasing heart weight. Data for normal hearts and hearts with acquired aortic stenosis (AS) are best approximated (r=0.93) as a third-order polynomial: y=3,599−8.45x+0.013x^2−6.76×10^{-6}x^3. Note that capillary density in hearts with congenital aortic stenosis or with aortic coarctation is always higher than expected from the above relation.](image)

however, was 2.5–2.9 times greater in hearts with aortic stenosis than in age-matched controls in all age groups.

Capillary density, capillary domain area, and the radius supplied by a single capillary were similar in control and corresponding hypertrophic hearts with congenital stenosis. Thus, the total number of coronary capillaries increased concordantly with the increase in cardiac weight, and the capillary density remained constant. SD log, an index of heterogeneity of capillary spacing, was higher in hypertrophic hearts, especially in the oldest group. An age effect on heterogeneity of capillary spacing with congenital aortic stenosis did not reach statistical significance by two-way ANOVA. The proportion of area occupied by myocytes and interstitium did not change with hypertrophy in the hearts with congenital aortic stenosis.

Adult-Onset Aortic Stenosis

Aortic stenosis acquired later in life was compared with congenital aortic stenosis in younger adults and with control hearts (Table 4). Results from adult congenital aortic stenosis were transferred from the data presented above (Table 3). The degree of hypertrophy was similar in both the congenital and acquired pressure-overload groups in Table 4 and was not measurably influenced by age. The capillarization of hearts with aortic stenosis acquired later in life, however, was significantly different from adult control hearts as well as hearts hypertrophied from aortic valve stenosis early in life (adult congenital aortic valve stenosis group). The coronary capillary density was significantly decreased (p<0.01), and both capillary domain area and radius of dependent perfusion were significantly greater in hearts with adult-onset aortic stenosis. Capillary heterogeneity (SD log) once again displayed a tendency toward higher values in hypertrophic hearts but did not reach levels of significance when all adult groups were analyzed together. When compared only with adult control hearts, however, hearts with acquired aortic stenosis displayed a significant increase in heterogeneity of capillary spacing (p<0.01). The relative proportion of the left ventricle occupied by interstitium remained unchanged.

Left Ventricular Hypertrophy With Coronary Hypertension in the Young Adult

Young adults with congenital coarctation and coronary hypertension did not differ from young adults with aortic valve stenosis and normal coronary artery pressure in the degree of hypertrophy, capillarization, or relative myocyte fraction of the left ventricle (Table 5).

Capillary Density as a Function of Cardiac Weight: Young Versus Old

Capillary density for each heart was plotted as a function of cardiac weight in Figure 2. Capillary density decreased exponentially with increasing cardiac weight in control hearts. The relation of capillary density and heart weight in hearts hypertrophic because of aortic stenosis acquired later in life is in direct continuation of the relation between capillary density and cardiac weight derived from normal hearts. The relation between capillary density and heart weight data in normal hearts of various ages and with acquired aortic stenosis is best approximated (r=0.93) as a third-order polynomial (Figure 2). In contrast, results obtained from cardiac hypertrophy caused by congenital aortic stenosis or coarctation lie above the line, indicating capillary densities higher than expected for a given heart weight. This was especially pronounced with cardiac hypertrophy in younger age groups.

The relation of the capillary domain area and heart weight data was qualitatively similar and best approximated (r=0.87) as a second-order polynomial (Figure...
TABLE 4. Heart and Capillary Morphometrics in Adult Human Hearts: Congenital Vs. Acquired Stenosis

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (kg)</th>
<th>Body surface area (m²)</th>
<th>Heart weight (g)</th>
<th>Capillary density (No/mm²)</th>
<th>Capillary domain area (μm²)</th>
<th>Capillary domain radius (μm)</th>
<th>SD log domain radius ×1,000</th>
<th>Myocyte fractional area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult control hearts (n=8)</td>
<td>58±3</td>
<td>1.61±0.04</td>
<td>255±24</td>
<td>2,249±85</td>
<td>449±17</td>
<td>12.0±0.2</td>
<td>59±1</td>
<td>82.1±0.9</td>
</tr>
<tr>
<td>Adult congenital aortic valve stenosis (n=7)</td>
<td>70±3</td>
<td>1.78±0.07</td>
<td>780±48*</td>
<td>2,102±103</td>
<td>482±22</td>
<td>12.4±0.3</td>
<td>73±14</td>
<td>82.4±1.3</td>
</tr>
<tr>
<td>Adult acquired aortic valve stenosis (n=13)</td>
<td>57±3</td>
<td>1.58±0.06</td>
<td>634±49*</td>
<td>1,671±66*</td>
<td>611±26*</td>
<td>13.9±0.3</td>
<td>67±2</td>
<td>80.6±0.4</td>
</tr>
</tbody>
</table>

SD log, standard deviation of the log radii. Values are mean±SEM. *p<0.01 compared with adult control group. †p<0.01 compared with adult congenital aortic valve stenosis.

3). The cross-sectional area of myocardium perfused by a coronary capillary was consistently smaller in hearts with congenital aortic stenosis or coarctation than predicted from the relation with heart weight seen in control hearts and acquired aortic stenosis.

Discussion

Animal Studies

A potential drawback to the study of pathology specimens was that the samples were fixed by immersion in formaldehyde. To test the validity of measurements from such specimens, capillary density in rat hearts fixed by glutaraldehyde perfusion were compared with results obtained from rat hearts fixed by immersion in glutaraldehyde or formaldehyde. Quantitative results of capillary density in samples fixed by immersion in formalin were similar to those of glutaraldehyde perfusion. The effect of other variables such as cardiac arrest in systole versus diastole and time from death to fixation could not be controlled for in this study. However, the capillary densities and range of capillary densities reported here are similar to previous studies in which these variables could be controlled. The human capillary densities measured by Roberts and Wearn28 in hearts perfused with fixative immediately after death were similar to those seen by us and others31 in human hearts fixed by immersion. All species of adult mammalian hearts studied have coronary capillary density in the same range, 2,000–4,000 capillary profiles per square millimeter of myocardium in cross section.13

Normal Human Maturation

Control patients in this series exhibited relatively normal somatic growth from infancy to young adulthood, with an approximately 10-fold increase in body weight and sixfold increase in heart weight. The left ventricular coronary capillary density in control hearts decreased with early postnatal cardiac growth and maturation, from 3,315 capillaries per square millimeter in infants to 2,388 capillaries per square millimeter in children, and thereafter remained relatively constant, averaging 2,249 capillaries per square millimeter in adults. (See also Figures 2 and 3.) The constancy of capillary density concordant with the 56% increase in heart weight between childhood and young adulthood suggests that coronary microvascular growth parallels the degree of cardiac myocyte growth between childhood and young adulthood. The decrease in coronary capillary density between infancy and childhood is similar to that measured in other mammalian species during early postnatal development.34 This finding contrasts with the conclusion stated by Roberts and Wearn29 in their classic study of human hearts that there were no developmental changes in capillary density, although it does agree with their own data showing a higher capillary density in the few infants measured.

The biological significance of the greater myocardial capillarity in neonates and infants is unknown. In neonates, the increase in capillarity may be a consequence of an adaptation of the fetus to a relatively hypoxic coronary circulation. In infants, greater myocardial capillarity may provide an adaptation to improve oxygen delivery in the presence of physiological anemia and increased myocardial oxygen consumption.35

In infant hearts, we observed a significantly higher percentage of space occupied by cardiac myocytes. This finding is consistent with the observation that the growth of nonmyocyte cells during postnatal development is much greater than the growth of myocytes.10,34 The fact that capillary numerical density also decreases after infancy implies an increase with age in relative space occupied by nonvascular myocardial interstitium. Capillary and interstitial volume were not directly measured, however. The age-related increase in normal blood pressure may have some influence on the left ventricular interstitial space, because elevations in blood pressure during adulthood can result in an increase of various interstitial components.13

TABLE 5. Capillary Morphometrics in Young Adults With Aortic Valve Stenosis (Normal Coronary Artery Pressure) Vs. Aortic Coarctation (Coronary Hypertension)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (kg)</th>
<th>Body surface area (m²)</th>
<th>Heart weight (g)</th>
<th>Capillary density (No/mm²)</th>
<th>Capillary domain area (μm²)</th>
<th>Capillary domain radius (μm)</th>
<th>SD log domain radius ×1,000</th>
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<td>780±48*</td>
<td>2,102±103</td>
<td>482±22</td>
<td>12.4±0.3</td>
<td>73±14</td>
</tr>
<tr>
<td>Adult congenital aortic coarctation (n=5)</td>
<td>65±8*</td>
<td>1.76±0.16</td>
<td>642±103</td>
<td>2,206±88</td>
<td>456±17</td>
<td>12.0±0.2</td>
<td>56±3</td>
</tr>
</tbody>
</table>

SD log, standard deviation of the log radii. Values are mean±SEM. *p<0.01 compared with adult control group.
Table 6. Summary of Morphometric Changes in Adult Patients With Left Ventricular Hypertrophy

<table>
<thead>
<tr>
<th></th>
<th>Congenital aortic stenosis</th>
<th>Congenital coarctation</th>
<th>Acquired aortic stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular mass</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Capillary density</td>
<td>→</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>Capillary domain</td>
<td>→</td>
<td>→</td>
<td>↑</td>
</tr>
<tr>
<td>Variability of capillary spacing</td>
<td>NS ↑</td>
<td>NS ↑</td>
<td></td>
</tr>
<tr>
<td>Myocytes, percent of area</td>
<td>→</td>
<td>→</td>
<td></td>
</tr>
</tbody>
</table>

↑, Increased; →, no change; ↓, decreased; NS ↑, statistically nonsignificant increase (by two-way ANOVA).

Capillary Density and Aortic Stenosis: Congenital Versus Acquired

These results are summarized in Table 6. In all age groups, infants, children, and adults, a sizable increase (almost threefold) in cardiac weight was noted in the pressure-overloaded hearts. Despite substantial cardiac hypertrophy, with congenital aortic stenosis, morphometric analysis did not reveal any changes in the relative distribution of coronary capillaries and cardiac myocytes. Hort and Severid31 also found no decrease in coronary capillary density from normal in several infants and children with a variety of congenital cardiac malformations and a mixture of right and left ventricular pressure overload, volume overload, and cyanosis.

Cardiac hypertrophy that developed in adult individuals was characterized by decreased capillary density and therefore a proportional increase in the cross-sectional area supplied by a single capillary, i.e., the capillary domain (Figure 1). A similar decrease of capillary supply in adult hypertrophic human hearts was reported by Roberts and Wearn.28 These data in humans are also similar to what has been observed in most studies of adult animals (e.g., see References 17–21 and 36–38), although animal studies have had some conflicting findings. For example, coronary conductance and microvascular density were normal in dogs with one-kidney, one-clip renal hypertension of 7 months duration29 but not with a shorter duration of 6 weeks.37 Similarly capillarity is normal with right ventricular hypertrophy of seven months duration.38 These animal studies suggested that in some models with moderate hypertrophy appropriate angiogenesis may occur with longer durations of pressure loading but seemed to trail the development of hypertrophy. Our study demonstrates that in adult humans with acquired hypertrophy of a much longer duration and greater degree than that seen in animal studies, normalization of the coronary capillary density fails to occur. This could impair oxygen transport to the myocardium8,10,17,18 and, as a result, contribute to the development of ventricular fibrosis,9,12,13 dysrhythmias,11,12 and impaired pump function observed in adults.41,42

Capillary density is only one of the geometric factors that influence oxygen supply to tissue.15,16 The heterogeneity of capillary spacing is also an important determinant of myocardial oxygenation. In previous studies in hearts of various mammalian species,17 we found an increased heterogeneity of capillary spacing in various types of experimental hypertrophy in animals. Results from human hearts, reported here for the first time, displayed a similar trend, with capillary spacing more heterogeneous in older subjects with aortic stenosis, but the differences failed to reach statistical significance when all groups were analyzed together.

Evidence for Age-Dependent Coronary Angiogenesis

Analysis of the relation of heart weight and coronary capillary density for all of the hearts further demonstrates the accelerated coronary growth response when pressure-overload hypertrophy develops in children in contrast with adults. Capillary density in young, normal hearts decreases with increasing cardiac weight, suggesting that the rate of capillary growth was less than that of cardiac growth in early childhood. Capillary density in hearts with increased cardiac mass as a result of aortic stenosis in adult patients seems to follow the same trend: The heart increases in weight because of an increase in size of the individual myocytes, and the capillary network becomes less dense. In contrast, left ventricular hypertrophy caused by congenital aortic stenosis is characterized by a different growth response: Left ventricular capillary density was higher than expected on the basis of the previous relation, apparently because of a different coronary angiogenic response. The mathematical curves used for this comparison were the best fit of the experimental data and were not intended to be direct mathematical models of the relations of capillary density and domain with heart weight. For the purpose of comparison, the use of the best fit of the data within a given range appears to be appropriate.

These results in humans indicating an effect of age on coronary capillarization with cardiac hypertrophy parallel our previous studies in experimental animals. Experimental aortic stenosis in young rabbits induced cardiomegaly with normal coronary microvascular volume, whereas the same stimulus in adult animals resulted in hypertrophic hearts with decreased capacity of microvasculature.24

Results showing an age-dependent effect of cardiac mass on coronary capillaries are consistent with the hypothesis17,24,34 that the growth response of coronary capillaries with development of myocardial hypertrophy is dependent on the age of the individual in whom the pressure overload is introduced. According to this hypothesis, coronary capillaries in the young proliferate as part of normal growth,34 and an additional increase in cardiac mass caused by pressure loading is accompanied by an accelerated, proportional growth of capillaries. In the adult, there is normally very little coronary capillary endothelial proliferation.34 With cardiac pressure-overload hypertrophy in the adult, there appears to be relatively less coronary capillary growth, resulting in diminished coronary capillary density. The precise mechanisms controlling coronary angiogenesis with development and hypertrophy are not yet known.

Because accurate measurement of arteriolar density was not possible in the pathology specimens used in this study, only capillary morphometry was studied. In animal studies of left ventricular pressure overload, the decrease in capillary density in adults is paralleled by similar decreases in arteriolar density.19,27 A decrease in arteriolar density could result in an additional decrease in oxygen transport capacity to the myocardium by limiting the coronary vascular blood flow capacity. Animal stud-
ies demonstrate that changes in coronary vascularity with pressure-overload hypertrophy at various ages are paralleled by qualitatively similar changes in coronary perfusion capacity.21,29,30 Coronary angiogenesis with pressure-overload hypertrophy in the young appears to be important in maintaining coronary capillary density and perfusion; pharmacological inhibition of angiogenesis during development of pressure-overload hypertrophy results in diminished capillary density accompanied by diminished coronary flow capacity.22 If the left ventricular arteriolar density in humans with aortic stenosis is diminished similarly to the capillary density, this alteration could be at least in part responsible for the diminishment in coronary flow reserve observed in adult patients with aortic stenosis.5-8

The limitation in coronary vascular reserve observed with hypertension in studies of adult animals,8,18,21,43 and in clinical studies of adult patients,44,45 appears to be more complex. Older children and younger adults with supravalvar aortic stenosis or coarctation have abnormal coronary flow,46,47 whereas the data presented here show normal coronary capillary density in subjects of similar age with aortic valvar stenosis and coarctation. Clinical and animal studies suggest that when pressure-overload left ventricular hypertrophy in adults is accompanied by coronary hypertension, there may be an additional diffuse adverse effect on coronary resistance vessels in both ventricles.18,21,27,38,43,45,46 as well as insufficient vascular growth, which may contribute to abnormalities in coronary perfusion.18,21,48 Supravalvar aortic stenosis and coarctation cause coronary systolic hypertension, which may result in abnormal thickening38,49 or vasomotion of the coronary resistance vessels.14,18 This has not been uniformly seen with coronary hypertension21,22,30,37,47 and is in contrast to isolated aortic valvar stenosis.7

Hypertension may lead not only to arteriolar thickening but also to a decrease in the absolute number of arterioles and capillaries in microvascular beds subjected to elevated pressure.40 Evidence for a decrease in the total number of coronary capillaries and arterioles has been seen in older animals with coronary hypertension.38 In this study, coronary capillary density in young adults with coarctation of the aorta was compared with normal hearts or valvar aortic stenosis to examine the effect of coronary hypertension. The coronary capillary density in hearts with coarctation was no different from that in normal hearts or those with valvar aortic stenosis. A net decrease in the number of coronary capillaries as a result of coronary hypertension was not apparent in humans at this age. Older children and young adults with severe aortic stenosis have exertional angina and ECG repolarization abnormalities and an incidence of sudden death as high as 8%.3 The data presented here show a normal coronary capillary density on average in patients with aortic stenosis who died at this age. Other mechanisms, for example, alterations in calcium currents resulting in afterdepolarizations51 and vasodepressor cardiac reflexes,52 may be involved in the clinical abnormalities seen in these patients.

In conclusion, detailed morphometric analysis of normal and hypertrophic human hearts disclosed several important results: 1) Capillary density values as well as the index of heterogeneity of capillary spacing (SD log) in human hearts are similar to those in other mammalian hearts examined. 2) Capillary density in human hearts normally decreases between infancy and mid-childhood. 3) Capillary growth with cardiac pressure-overload hypertrophy is dependent on the age at which the growth stimulus occurs. Cardiac hypertrophy developing in the adult heart is characterized by a decreased capillary density. In contrast, left ventricular hypertrophy caused by congenital aortic valve stenosis demonstrates a capillary density similar to that of control hearts of much smaller weight. Thus, pressure-overload hypertrophy with onset in childhood is structurally different from cardiac hypertrophy originating in adult patients.

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