Capillary Geometrical Changes With Fiber Shortening in Rat Myocardium

David C. Poole, Sanjay Batra, Odile Mathieu-Costello, and Karel Rakusan

Capillary-to-fiber geometrical relations constitute an integral component of peripheral gas exchange. Determination of capillary length and surface area density necessitates quantification of capillary orientation (i.e., tortuosity and branching). In skeletal muscle, capillary tortuosity increases in a curvilinear fashion at reduced sarcomere length, and this compensates for decreased capillary density as fiber cross-sectional area increases. To investigate these relations in myocardium, rat hearts were glutaraldehyde perfusion-fixed in calcium- or barium-induced “systole” to provide varying degrees of fiber shortening. Morphometric techniques were used to analyze capillary geometry in subepicardium (EPI) and subendocardium (ENDO) using 1-μm sections cut transverse and longitudinal to the muscle fiber axis. Capillary density on transverse and longitudinal sections, capillary diameter, fiber cross-sectional area, and sarcomere length were determined in each region. Capillary surface density was computed, and values were related to sarcomere length and compared with published data for diastolic hearts. Sarcomere length in systole ranged from 2.06±0.03 to 1.35±0.02 μm (EPI) and from 1.93±0.04 to 1.44±0.04 μm (ENDO). Fiber cross-sectional area (EPI, 344±13 μm²; ENDO, 343±12 μm²) was significantly larger, and capillary density on transverse sections was significantly smaller (EPI, 4,105±318 mm⁻²; ENDO, 4,145±267 mm⁻²) than in hearts arrested in diastole. Compared with skeletal muscle, capillary tortuosity was substantially less increased by fiber shortening. Capillary tortuosity and branching did not differ between EPI and ENDO and contributed a maximum of 33% (range, 13–33%) to capillary length density and surface area at a sarcomere length of 1.45±0.04 μm. Compared with diastolic hearts, capillary length density decreased on average by 19.6% (EPI) and 17.7% (ENDO); similarly, capillary surface density decreased 19.9% (EPI) and 13.7% (ENDO). We conclude that, with fiber shortening in the heart, fiber cross-sectional area increases and capillary numerical density decreases as predicted from reduced sarcomere length. Combined with the minimal geometrical changes of the capillary bed at shorter fiber lengths, this results in a lower capillary length and surface area per fiber volume in systole. Consequently, the structural potential for O₂ diffusion into myocytes is determined, in part, by fiber length. (Circulation Research 1992;70:697–706)

KEY WORDS • systole • diastole • sarcomere length • myocardial oxygenation • gas exchange • capillary density

In heart, it is widely held that the capillary bed is highly oriented with the long axis of the muscle fibers.¹⁻⁵ Capillaries are depicted generally as long straight vessels with some degree of branching.⁶⁻⁸ Morphometric measurements have shown a variable but modest contribution of capillary tortuosity and branching to capillary length per fiber volume in left ventricle²,⁴,⁵,⁹ and capillary muscle.¹⁰ However, no investigation has yet related the geometrical disposition of the capillary bed to fiber and sarcomere length during different phases of the cardiac cycle.

Studies of capillary geometry are necessary to adequately describe the gas exchange potential of the capillary bed. This consideration assumes particular importance in striated muscle, where capillary geometry changes profoundly with alterations in sarcomere length.¹¹⁻¹³ Specifically, as sarcomere length is reduced below 2.0–2.4 μm, capillaries become tortuous, and the contribution of this component to capillary length and surface area increases in a curvilinear fashion. This behavior compensates for the reduced capillary density consequent to augmented fiber cross-sectional area, and capillary length and surface area are practically unchanged below 2.0–2.4 μm.¹⁴ The dependence of fiber cross-sectional area, capillary density, and capillary tortuosity on sarcomere length underscores the importance of referencing these measurements to sarcomere length.¹⁵

Techniques developed for quantification of capillary tortuosity and branching in skeletal muscle have been used to quantify capillary geometry in the myocardium.⁵,⁹ In the left ventricle of hearts perfusion-fixed in diastole, capillary tortuosity and branching contributed 6–27% to capillary length above that calculated on the

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assumption that capillaries are straight, unbranched structures oriented perfectly parallel to the muscle fibers. As expected for hearts fixed in diastole, the range of sarcomere lengths was not large (from 2.09±0.02 to 2.23±0.02 µm in subepicardium and from 2.04±0.03 to 2.17±0.02 µm in subendocardium; see Reference 9). This precluded exploration of the relation between sarcomere length and capillary geometry in that investigation. It is not known whether capillary orientation changes with altered sarcomere length in myocardium as is the case for mammalian skeletal muscle. Intriguingly, photomicrographs of capillaries in hearts considered to be in systole demonstrated highly oriented vessels.6,7 Unfortunately, the relation between capillary orientation and sarcomere length was not explored.

Barium-induced fiber shortening has recently been used to evaluate rat myocardial blood volume reductions in large (>100-µm) and small (<100-µm) vessels during systole.16 During myocardial fiber shortening, there are two mechanisms by which capillary (or larger vessel) volume may be decreased: 1) complete or partial vessel collapse (reduced cross-sectional area) and 2) reduced capillary length. The purpose of this investigation was to determine the relation between capillary geometry and sarcomere length during fiber shortening in the heart. In turn, these data permitted quantification of changes in capillary length and surface area per fiber volume (i.e., determinants of blood–tissue exchange potential). Results from hearts analyzed in “systole” were compared with hearts perfusion-fixed in diastole.9

Materials and Methods

Tissue Preparation

Female Sprague-Dawley rats (body weight, 197–237 g; n=6) were anesthetized with sodium pentobarbital (4 mg/100 g i.p.). After catheterization of the iliac vein, the animals were heparinized (0.3 ml, 10,000 units·ml−1) and injected with papaverine (0.3 ml, 30 mg·ml−1). Immediately after these procedures, a bolus injection of either calcium chloride (rats 1–4) or barium chloride (rats 5 and 6) was administered to induce sustained contraction. We defined this condition as “systole,” recognizing that the sustained contraction thus produced is not true physiological systole. The chest was opened, and the heart was perfused via the aorta either in situ (rats 1–4 and 6) or while suspended in vitro (rat 5). The right atrium was cut for outflow, and hearts were perfused with saline at a nonpulsatile pressure of 90–110 mm Hg, until the exudate was clear of cells (1–4 minutes). The saline contained 30 mM CaCl2 or BaCl2 adjusted to 350 mosm with NaCl. Subsequently, the heart was perfused with glutaraldehyde fixative (i.e., 6.25% glutaraldehyde solution in 0.1 M sodium cacodylate buffer adjusted to 430 mosm with NaCl; total osmolarity of fixative, 1,100 mosm; pH 7.4) for 4–5 minutes. Directly after perfusion with glutaraldehyde fixative, the heart was excised and weighed, and a sample (~4 mm long by 3 mm wide, entire thickness of ventricle wall) was taken from the mid portion of the left ventricular free wall, ~70% of distance from the apex on the apex-to-base axis. The tissue was subsampled into subepicardial and subendocardial portions of equal size, and the remaining midwall section (~0.2 mm thick) was discarded. Subepicardial and subendocardial portions were subsequently cut into 20 or more blocks, immersed in glutaraldehyde fixative, and processed for electron microscopy using standard techniques.11

Tissue Sectioning

Sections 1 µm in thickness were cut using an LKB Ultratome III (Leica, Inc., Deerfield, Ill.) and stained with 0.1% aqueous toluidine blue solution. Of the 20 blocks from each subepicardial and subendocardial sample, eight blocks were cut to obtain longitudinal and transverse sections. The method used to obtain sections taken rigorously parallel or perpendicular to the muscle fiber axis is detailed below.11 Each block is positioned in the specimen holder of the microtome in a preferred orientation (i.e., approximately transverse or longitudinal to the muscle fiber axis). Consecutive sections are then taken at different angles with respect to the fiber axis by systematically changing the specimen holder orientation by 1° and 5° for longitudinal and transverse sections, respectively. Sections are defined as transverse when a change of sectioning angle by 5° in either direction produces fiber sections with a smaller A-band spacing. Longitudinal sections are identified when a change of sectioning angle of 1° in either direction gives fiber sections with greater sarcomere length.11

Compared with skeletal muscles, additional technical difficulties present themselves because of the mixed orientation of cardiac fibers.9 It was not always possible to position the block in the specimen holder in a position that was preferential with respect to the orientation of all the muscle fibers. Rather, an initial section was taken, often containing fibers that were cut approximately transverse and others that were approximately longitudinal or oblique to the fiber axis. A region showing either longitudinally or transversely cut fibers was then chosen, and the procedure described above (systematic change of specimen holder orientation) was followed. It was routinely necessary to take 15 or more sections to obtain an adequate number of fibers (20–100) for analysis.

Morphometric Analysis

Morphometric data were collected from the subepicardium and subendocardium of six hearts. Photomicrographs (35-mm direct positive, Kodak Direct MP 5360) were taken of those regions that fulfilled the criteria for sections taken either transverse or longitudinal to the muscle fiber axis using a Leitz Ortholux light microscope equipped with a Leica camera. A stage micrometer was photographed with each film for calibration. Within each region, all fibers were subsampled systematically to yield as many nonoverlapping micrographs as possible. The total number of micrographs ranged from 22 to 76 (43±2 [mean±SEM]) and from 15 to 33 (26±2) micrographs per region for longitudinal and transverse sections, respectively, yielding from 530 to 1,100 (814±39) fibers per subepicardium or subendocardium in transverse sections and from 170 to 290 (235±56) longitudinal portions of muscle fibers in longitudinal sections.

The films were projected onto a square grid (144 points) at a final magnification of x2,060 by means of a Documator (model DL2, Jenoptik, Jena, FRG) microfilm reader. Point-counting was used to determine
Capillary numerical and volume density. Points were collected, stored, and processed using an Apple II+ computer. Standard errors of the estimates of capillary densities were calculated by pooling data from all micrographs from one preparation and applying formulas for the standard error of ratios. The standard error is therefore a measure of the between-micrograph variability at the sampling site analyzed in each preparation and reflects the degree of biological variability in each region. The group mean standard error indicates the biological variability between hearts. This was below 10% in all instances.

Capillary length per volume of muscle fiber \(J_v(c,f)\), the degree of capillary orientation concentration parameter (K), and the proportional contribution of the anisotropic components (tortuosity and branching) to capillary length per fiber volume \(c(K,0)\) were estimated using the method developed by Mathieu et al. Briefly, the method is as follows: We have demonstrated previously that the Fisher axial distribution model is suitable for estimation of capillary anisotropy in rat heart. Accordingly, \(J_v(c,f)\) is related to capillary numerical density in transverse sections \(Q_A(0)\) and longitudinal sections \(Q_A(\pi/2)\) by the following equations:

\[
J_v(c,f) = c(K,0) \cdot Q_A(0)
\]

\[
J_v(c,f) = c(K,\pi/2) \cdot Q_A(\pi/2)
\]

where \(c(K,0)\) and \(c(K,\pi/2)\) are anisotropy coefficients for transverse and longitudinal sections, respectively.

Combining and rearranging Equations 1 and 2 gives

\[
Q_A(0)/Q_A(\pi/2) = c(K,\pi/2)/c(K,0)
\]

The ratio \(c(K,\pi/2)/c(K,0)\) in the Fisher axial distribution model is a uniform and monotonic function of K. Thus, both \(c(K,0)\) and \(J_v(c,f)\) can be estimated by the following procedure: capillary numerical density is determined on transverse \(Q_A(0)\) and longitudinal \(Q_A(\pi/2)\) sections (four blocks each). This gives the ratio \(Q_A(0)/Q_A(\pi/2)\) (Equation 3), which is used to determine K and \(c(K,0)\) from a table (or graph) of known coefficients. Then, \(J_v(c,f)\) is estimated via Equation 1 (or 2).

For straight capillaries oriented perfectly parallel to the muscle fibers, \(K = \pi\) and \(c(K,0) = 1\); for randomly oriented (isotropic) capillaries, \(K = 0\) and \(c(K,0) = 2\).

As in previous studies (e.g., Reference 11), all estimates of capillarity were expressed using the muscle fibers as the reference space in each sample. This procedure avoids any bias resulting from unreliable preservation of the extracellular space. Capillary-to-fiber ratio \(N_v(c,f)\) and capillary volume density \(V_v(c,f)\) were estimated directly using standard point-counting procedures on transverse sections. Fiber cross-sectional area was measured with an image analyzer (Videometric 150, American Innovation Inc., San Diego, Calif.) on transverse sections. Capillary diameter was measured by image analysis on over 100 capillaries per subepicardial or subendocardial sample, from circular profiles only (difference between minimum and maximum diameters, <15%). Capillary surface per volume of muscle fiber \(S_v(c,f)\) gives the surface area available for blood–tissue exchange. According to Fick’s Law, the capacity for \(O_2\) diffusion will be determined in part by the available surface area, i.e., \(S_v(c,f)\), which is given by

\[
S_v(c,f) = J_v(c,f) \cdot \pi \cdot d(c)
\]

where \(d(c)\) is capillary diameter.

**Tissue Shrinkage**

The preparation procedures used produce a certain amount of tissue shrinkage. Although this occurs to some degree in both transverse and longitudinal directions, the capillary geometry herein described would be affected principally by longitudinal shrinkage (i.e., filament length changes). Data from our laboratory for rat diaphragm muscle indicate that glutaraldehyde fixation and subsequent processing as herein described decrease actin filament length by ~6% (D.C. Poole and O. Mathieu-Costello, unpublished observations, April 1991). In the present investigation we elected not to correct values of sarcomere length for this modest shrinkage.

**Statistical Analysis**

Differences between subepicardium and subendocardium of the same heart and between different hearts were assessed by means of unpaired t tests. Correlation analyses were performed using standard least-squares regression techniques. Significance was accepted at \(p<0.05\).

**Results**

The macroscopic appearance of equatorial sections of hearts perfusion-fixed either in diastole or at two levels of fiber shortening is shown in Figure 1. At an intermediate level of fiber shortening, sarcomere length averaged 2.06±0.03 and 1.93±0.03 \(\mu m\) in subepicardium and subendocardium, respectively (Figure 1, middle panel; heart 1, Table 1). An example of complete closure of the ventricular cavities (extreme fiber shortening) is also shown (Figure 1, right panel; heart 2, Table 1). For this heart, sarcomere length averaged 1.43±0.16 and 1.64±0.10 \(\mu m\) in subepicardium and subendocardium, respectively. The range of sarcomere lengths in all hearts analyzed in calcium- or barium-induced systolic was from 2.06±0.03 to 1.35±0.02 \(\mu m\) for subepicardium and from 1.93±0.04 to 1.44±0.04 \(\mu m\) for subendocardium (Table 1). For comparison, corresponding values for hearts previously fixed in diastole were 2.16±0.04 (subepicardium) and 2.09±0.04 \(\mu m\) (subendocardium). For \(CaCl_2\) or \(BaCl_2\) contraction-induced systolic (this study) and diastolic hearts, mean sarcomere length in subepicardium (1.83±0.11 \(\mu m\), \(n=10\)) was highly correlated \((r=0.919, p<0.001)\) and not significantly different from that in subendocardium \((1.82±0.08 \mu m)\).

There was no significant difference between subepicardium and subendocardium for any of the morphometric variables measured in this investigation (Tables 1 and 2). Fiber cross-sectional area \(\tilde{A}(f)\) ranged from 291±34 to 384±14 \(\mu m^2\) in subepicardium and from 302±27 to 381±40 \(\mu m^2\) in subendocardium (Table 1). The values for “systolic” hearts are compared with hearts fixed in diastole in Figure 2, top panel. The increased \(\tilde{A}(f)\) in both regions of “systolic” hearts is consistent with that predicted on the basis of the reduced sarcomere length (\(l\))
TABLE 1. Morphometric Data for Subepicardium and Subendocardium of “Systolic” Rat Heart

<table>
<thead>
<tr>
<th>Rat heart No.</th>
<th>(l) ((\mu\text{m}))</th>
<th>(Q_A(0)) ((\text{mm}^2))</th>
<th>(Q_A(\pi/2)) ((\text{mm}^2))</th>
<th>(K)</th>
<th>(c(K,0)) (mm)</th>
<th>(J(c,f)) ((\text{mm}^2))</th>
<th>(\bar{a}(f)) ((\mu\text{m}^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subepicardium</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.06±0.03</td>
<td>5,445±172</td>
<td>1,821±57</td>
<td>2.36±0.13</td>
<td>1.18±0.02</td>
<td>6,425±231</td>
<td>291±24</td>
</tr>
<tr>
<td>2</td>
<td>1.43±0.16</td>
<td>4,209±178</td>
<td>1,794±69</td>
<td>1.72±0.13</td>
<td>1.28±0.03</td>
<td>5,388±259</td>
<td>342±19</td>
</tr>
<tr>
<td>3</td>
<td>1.53±0.18</td>
<td>4,022±121</td>
<td>1,516±53</td>
<td>2.02±0.12</td>
<td>1.22±0.02</td>
<td>4,907±167</td>
<td>352±12</td>
</tr>
<tr>
<td>4</td>
<td>1.35±0.02</td>
<td>4,227±119</td>
<td>1,638±35</td>
<td>1.95±0.13</td>
<td>1.24±0.02</td>
<td>5,241±168</td>
<td>329±48</td>
</tr>
<tr>
<td>5</td>
<td>1.85±0.03</td>
<td>3,605±166</td>
<td>1,253±57</td>
<td>2.25±0.18</td>
<td>1.19±0.03</td>
<td>4,290±223</td>
<td>384±14</td>
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<tr>
<td>6</td>
<td>1.45±0.04</td>
<td>3,121±113</td>
<td>1,458±62</td>
<td>1.51±0.13</td>
<td>1.33±0.03</td>
<td>4,151±178</td>
<td>365±23</td>
</tr>
<tr>
<td>Mean±SEM (n=6 hearts)</td>
<td>1.61±0.11</td>
<td>4,105±318</td>
<td>1,580±88</td>
<td>1.97±0.13</td>
<td>1.24±0.02</td>
<td>5,067±338</td>
<td>344±13</td>
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<td><strong>Subendocardium</strong></td>
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</tr>
<tr>
<td>1</td>
<td>1.93±0.04</td>
<td>5,021±134</td>
<td>1,625±62</td>
<td>2.46±0.15</td>
<td>1.17±0.02</td>
<td>5,875±188</td>
<td>329±20</td>
</tr>
<tr>
<td>2</td>
<td>1.64±0.10</td>
<td>3,640±121</td>
<td>1,682±52</td>
<td>1.53±0.11</td>
<td>1.32±0.03</td>
<td>4,807±192</td>
<td>364±20</td>
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<tr>
<td>3</td>
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<td>4,035±138</td>
<td>1,405±83</td>
<td>2.24±0.21</td>
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<td>361±11</td>
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<tr>
<td>4</td>
<td>1.44±0.04</td>
<td>3,931±198</td>
<td>1,119±39</td>
<td>2.97±0.29</td>
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<td>5</td>
<td>1.59±0.10</td>
<td>4,845±170</td>
<td>1,520±73</td>
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<td>1.16±0.02</td>
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<tr>
<td>6</td>
<td>1.55±0.04</td>
<td>3,391±118</td>
<td>979±49</td>
<td>2.85±0.25</td>
<td>1.14±0.02</td>
<td>3,866±151</td>
<td>381±40</td>
</tr>
<tr>
<td>Mean±SEM (n=6 hearts)</td>
<td>1.64±0.07</td>
<td>4,145±267</td>
<td>1,388±115</td>
<td>2.42±0.20</td>
<td>1.19±0.06</td>
<td>4,904±304</td>
<td>343±12</td>
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</tbody>
</table>

Values are mean±SEM for within-heart samples for each rat. \(l\), Sarcomere length; \(Q_A(0)\), capillary counts per fiber area (\(\text{mm}^2\)) on transverse sections; \(Q_A(\pi/2)\), capillary counts per fiber area (\(\text{mm}^2\)) on longitudinal sections; \(K\), concentration parameter of Fisher axial distribution; \(c(K,0)\), index of contribution of capillary tortuosity to total capillary length; \(J(c,f)\), capillary length per volume of muscle fiber; \(\bar{a}(f)\), mean fiber cross-sectional area.

and assumed constant fiber volume,\(^{15,20}\) according to the following relation:

\[
\bar{a}(f)_m = \bar{a}(f)_p \cdot \left( \frac{l_m}{l_p} \right)
\]


Figure 1. Macroscopic appearance of equatorial sections of perfusion-fixed hearts. Left panel: Diastolic heart. Center panel: Midrange fiber shortening. Right panel: Extreme fiber shortening. Scale is in centimeters.
length (-0.1142±0.0546) achieved a significance level of 0.051 (n=20, Figure 3). The mean capillary orientation concentration parameter K (subepicardium, 1.97±0.13; subendocardium, 2.42±0.20) was also not significantly different from that in diastolic hearts (subepicardium, 3.28±0.90; subendocardium, 3.20±0.60, from Reference 9) but yielded a slope that was different from 0 when plotted against sarcomere length (1.49±0.67, p<0.05). For data from “systolic” and diastolic hearts (n=10), K was significantly correlated between subepicardium and subendocardium (r=0.872, p<0.05). This was also true for c(K0) (r=0.637, p<0.05). For comparison, c(K0) for rat hind limb muscle (dotted line, Figure 3) at 1.6 μm sarcomere length was -1.7.

Capillary length per fiber volume, Jv(c,f), averaged 5,067±338 mm⁻² in subepicardium and 4,904±304 mm⁻² in subendocardium (Table 1). In “systolic” hearts, the additional capillary length contributed by tortuosity and branching ranged from a minimum of 475 mm⁻² (heart 6, subendocardium) up to 1,179 mm⁻² (heart 2, subepicardium) and averaged 861±66 mm⁻² in all samples [compare Jv(c,f) with Qv(0) in Table 1]. Compared with hearts fixed in diastole (data from Reference 9), the value of Jv(c,f) was 19.6% smaller (subepicardium) and 17.7% smaller (subendocardium) in “systolic” hearts. The plot of Jv(c,f) against sarcomere length is given in Figure 4. Because capillary diameter (for “systole”: subepicardium, 4.90±0.12 μm; subendocardium, 4.71±0.12 μm; for diastole: subepicardium, 4.91±0.26 μm; subendocardium, 4.46±0.22 μm) did not change as a function of sarcomere length, the effect of changed sarcomere length on capillary surface area density, Sv(c,f), was similar to that of Jv(c,f). In “systole,” Sv(c,f) decreased an average of 19.9% in subepicardium and 13.7% in subendocardium. It is notable that capillaries were fixed open (i.e., plasma

<table>
<thead>
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<tr>
<td>Rat heart No.</td>
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<tr>
<td>Subepicardium</td>
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<td>Mean±SEM (n=6 rat hearts)</td>
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<td>Subendocardium</td>
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<tr>
<td>Mean±SEM (n=6 rat hearts)</td>
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</table>

Values are mean±SEM for within-heart samples for each rat. Nv(c,f), capillary-to-fiber ratio; Vv(c,f), capillary volume density; d(c), mean capillary diameter; Sv(c,f), capillary surface area per volume of muscle fiber; b(f), fiber perimeter.

FIGURE 2. Graphs showing relation between sarcomere length and fiber cross-sectional area and capillary density (i.e., capillary number per square millimeter of myocyte sectional area) for hearts fixed in “systole” (solid symbols) or diastole (hollow symbols). Top panel: Solid curve is theoretical relation for a fiber of 305 μm² (at 1.8 μm) over the range of sarcomere lengths given. Bottom panel: Solid curve is for tissue sample with 4,300 capillaries/mm² (at 1.8 μm) over the range of sarcomere lengths given. Both theoretical curves assume constant fiber volume.
surrounding erythrocytes), despite the frequent presence of erythrocytes in the lumen.

The capillary-to-fiber ratio, N_s(f,c), averaged 1.33±0.08 in subepicardium and 1.32±0.08 in subendocardium. These values compare closely with those obtained in diastolic subepicardium (1.28±0.08) and subendocardium (1.30±0.05).9

**Discussion**

One principal finding of the present investigation is that increases of capillary tortuosity with myocardial fiber shortening are minimal, compared with those in skeletal muscle below sarcomere lengths of 2.0–2.4 μm.11–13

**What Is the Physiological Range of Myocardial Sarcomere Lengths?**

As stated previously, the purpose of this investigation was to determine whether changes in capillary geometry accompany fiber shortening in the heart. Allowing that the condition of sustained contraction necessary to facilitate perfusion fixation at reduced fiber sarcomere lengths may not represent true physiological systole, it is important to relate the sarcomere lengths obtained in this investigation to those that might occur in vivo. Whereas skeletal muscles can produce active tension over a broad range of sarcomere lengths,21–23 in vivo muscles may only operate over that portion of their length–tension relation where active tension production is close to optimal. This has been demonstrated in a wide variety of muscles (e.g., in carp red muscle during swimming,24 in flight muscles of birds during flight,25 and in human thigh muscles during walking26). Cardiac muscle produces less force per unit cross-sectional area than skeletal muscle, but the shape of the length–active tension relation is similar.27 However, in contrast to skeletal muscle, the ascending limb of the length–tension curve has been considered the only physiologically functional region in the heart.28 Extreme sarcomere length values of 1.4 and 2.4 μm have been reported for cat papillary muscle.29 However, the normal limits of the ascending limb have been considered as 1.5 and 2.2 μm.27–28,30 These values correspond well with laser diffraction measurements of sarcomere length over the ascending limb of the length–active tension curve in rat trabeculae at different Ca2+ concentrations.22 In pathological conditions such as postischemic contracture and reperfusion, mean values as low as 1.25 μm have been reported for the left ventricle.31

At normal left ventricular end-diastolic pressure of 12 mm Hg, sarcomere lengths in subepicardium and subendocardium averaged 2.180±0.001 and 2.120±0.10 μm, respectively.32 Sommenblick et al30 estimated that sarcomere length in systole will shorten to ~1.7 μm at physiological afterload. If it were appropriate to consider a spherical geometry, a circumferential fiber shortening of this magnitude could account for a 40–50% ejection fraction.30 It was further demonstrated that during postextrasystolic potentiation, sarcomere length shortened to 1.6 μm, predicting an ejection fraction of ~75%.33

Studies of left ventricular volumes and ejection fraction in humans have used radionuclide ventriculography at rest and during maximal or near maximal upright cycle ergometry. Ejection fraction at rest for young adult males and females averaged 64–72% and increased significantly to 79–91% on exercise.34–36 Although the change of end-diastolic volume during exercise was variable, a consistent feature was a decreased end-systolic volume (by 36–65%) from rest to exercise. If morphometric and physiological data in animals (rats, cats, and dogs) can be compared with those in humans, this suggests that myocardial sarcomere length during maximal exercise may decrease below 1.6 μm.

In summary, we are reasonably confident that the sarcomere lengths found in diastole (i.e., up to 2.23±0.02 μm, from Reference 9) and “systole” (down to 1.35±0.02 μm, Table 1) incorporate, if not exceed, the range of sarcomere lengths attained in myocardium in vivo. Recognizing that it is not possible to put a minimum value on sarcomere length in vivo, the value of 1.35±0.02 μm at the lower extreme is likely beyond that found under physiological as opposed to pathological conditions (e.g., postischemic contracture).30

**Regional Variation in Sarcomere Length**

Variability of sarcomere length may occur at different levels, i.e., within fiber, between adjacent fields within a

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**Figure 3.** Graph showing relation between sarcomere length and the capillary anisotropy coefficient [c(K,0)]. Solid symbols denote “systolic” hearts, and hollow symbols are diastolic heart data from Reference 9.

**Figure 4.** Plot of capillary length per fiber volume against sarcomere length. Solid symbols are “systolic” hearts; hollow symbols are diastolic heart data from Reference 9.
region, across the myocardial wall, and from apex to base.

Within fiber. Using computer-interfaced optical microscopy, Roos37 derived three-dimensional reconstructions to determine sarcomere length and registration throughout the volume of individual rat heart myocytes. With the exception of regions of sarcomere distortion close to the nuclei, sarcomere registration and length periodicity exhibited good uniformity throughout the cell. In the present investigation, sarcomere lengths could often be determined over the entire length of a given myocyte and variability was greater than that observed previously in diastole. For “systolic” hearts, the coefficient of variation (CV) for sarcomere length within fibers averaged 12.6±1.8% (n=28).

Adjacent fields. Variation between fibers in the same field are reportedly small (CV=−2.3%, from Reference 28). On average, we found CV=7.4±1.9% (n=12). This was greater than in hearts fixed in diastole (CV up to 2.3%, with an average of 1.0±0.5%, n=8, from Reference 9).

Across wall. Sarcomere length differences across the left ventricular wall may be dependent on intraventricular pressure or volume.28 However, these differences are typically small, and their directional bias is controversial.28-30,32 Furthermore, at intraventricular pressures of ~20 mm Hg, they are largely abolished. In the present investigation, no consistent differences were found between sarcomere length in subepicardium and subendocardium (Table 1).

Base to apex. Differences in sarcomere length have been reported between the left ventricular apex and base of the dog heart.28 We chose to minimize such variability by sampling all hearts in precisely the same location in the left ventricular free wall.

To summarize, some degree of sarcomere length heterogeneity exists at each level considered. In the present investigation, the larger variability in “systolic” hearts likely reflects changes in fiber orientation associated with systole and/or the chemically induced sustained fiber shortening. However, the strong correlation between sarcomere length in subepicardium and subendocardium (r=0.919, p<0.001) suggests that the mean values obtained in each region are valid and adequately describe sarcomere and hence fiber length in any particular heart or region.

Fiber Cross-sectional Area and Capillarity

Because myocardial fiber cross-sectional area, a(f), changes reciprocally with sarcomere length, capillary density, Qc(0), is expected to decrease with decreased sarcomere length. Using the data obtained in diastolic rat heart,28 we predict (Equations 5 and 6) a(f) and Qc(0) values of 362±39 μm² and 4,114±529 mm⁻², respectively, at 1.61 μm sarcomere length. These values are not significantly different from those found in Table 1 [a(f), 344±13 μm²; Qc(0), 4,105±318 mm⁻²]. Similarly, we predict a(f) and Qc(0) values of 362±20 μm² and 4,110±221 mm⁻², respectively, in the subendocardium at 1.64 μm sarcomere length, based on previous findings in diastolic hearts.28 Again, these values are not significantly different from those measured in “systolic” hearts (Table 1 and Figure 2). These values, together with the unchanged capillary-to-fiber ratios, Nc(f), between the two groups (Table 2 and Reference 9), support the validity of the techniques used to determine a(f) and capillarity in this investigation. For instance, had changes in left ventricular geometry impaired our ability to secure fibers cut transversely, measured a(f) would have exceeded that predicted. Similarly, if capillaries had for some reason not been counted (e.g., not detected because of lumen collapse) in the “systolic” hearts, then Nc(f) would be expected to fall from that found in diastole. The data suggest strongly that these putative sources of error were negligible or absent. However, it is important to note that the relations of a(f) and Qc(0) with sarcomere length are evident only when the whole range of sarcomere lengths is considered. Within “systolic” or diastolic groups, there is substantial variability at a given sarcomere length. This is likely, in part, to be a consequence of the biological variability between hearts.

If capillary density (i.e., number per square millimeter of fiber cross-sectional area) is lower in “systolic” and capillary length does not become manifest as increased tortuosity, then capillary length, Jv(c,f), volume, Vv(c,f), and surface density, S(c,f), must fall. Compared with diastolic hearts, this investigation demonstrates that the value of these variables was on average 19.6%, 36.4%, and 20.0% smaller, respectively, in “systolic” subepicardium and 17.7%, 26.4%, and 13.7% smaller, respectively, in “systolic” subendocardium. This is consistent with the findings of Judd and Levy16 that the microvasculature undergoes large volumetric changes with fiber shortening. It extends those measurements to consider capillary length and surface area changes and to relate these to changes in sarcomere length. It also permits consideration of the effects of decreased capillary surface area on blood–tissue gas exchange. For instance, if we consider that the capillary-to-fiber interface constitutes an important barrier for O₂ diffusion within the heart,29 Fick’s law predicts that at reduced fiber lengths decreased capillary surface area will compromise oxygen delivery to the myocytes irrespective of altered blood flow.

Mechanical Behavior of Capillaries

Two particularly remarkable facets of the systolic capillary bed were evident in this investigation: 1) At a given sarcomere length (<~2.0 μm), the degree of orientation of capillaries was markedly higher than that reported in mammalian skeletal muscles.12,13 2) Despite the high intramuscular pressures reported for subendocardium during systole,40 capillaries remained open in this region.

With regard to the reduced development of capillary tortuosity in cardiac muscle, at least two structural features of the capillary bed are relevant. First, collagen struts extend from the capillary basement membrane to all contiguous myocytes. These struts are wavy in diastole but become taut in systole,41,42 and it is conceivable that they constrain lateral movement of the capillary and interfere with the development of tortuosity during fiber shortening. Second, the high density of capillary branches found in the heart may reduce capillary deformation. This hypothesis is supported by the similar c(F,0)-to-sarcomere length relation in pigeon pectoralis muscle, which also exhibits a prodigious number of capillary branches running perpendicular to the muscle fiber axis.43
The capillary-to-myocyte collagen struts mentioned above may also be important in preventing capillary lumenal closure during systole. Wiggers and Caulfield and Borg have suggested that these struts are responsible for the subendocardium sustaining some capacity for flow during systole. Alternatively, it is possible that tissue compression collapses extracapillary vessels and thus capillary lumenal pressure rises with intramuscular pressure. In agreement with this, the presence of plasma around erythrocytes in capillaries indicates that even nonperfused capillaries do not always collapse. Further support for this notion comes from the work of Judd and Levy, who demonstrated that, during barium-induced systole, blood volume in midwall and subendocardial vessels >100 μm diameter fell to zero. Because barium-induced systole may incur higher tissue pressures than physiological systole, it is possible that in vivo not all larger (>100-μm) vessels are collapsed. In this instance, a dynamic equilibrium would be achieved where narrowing incurred by partial collapse had generated adequate proximal intraluminal pressure to prevent further closure downstream. Thus, as long as intraluminal pressure equals or exceeds tissue pressure, the vessel will not collapse, and the outflow orifice will be reduced sufficiently in size to generate this pressure. Consequently, flow will be set by the interaction of the two pressures and not depend on the upstream intraluminal diameter. This effect has been described as a “vascular waterfall.” In conclusion, our observations (i.e., capillary lumen open with or without erythrocytes present) and those of others are consistent with the increased restriction to blood flow being located primarily in the extracapillary vessels during “systole.”

Red Blood Cell Transit Time

One potential benefit of either maintaining erythrocyte flux or at least volume in subendocardium during systole is that mean cell transit time would be increased. If we consider the rat heart during near-maximum exercise, coronary blood flow = 8.2 ml·g⁻¹·min⁻¹ (measured using microspheres injected into the left ventricle); subendocardial/subepicardial flow ratio = 1.03 (measured in dogs); capillary volume in diastole, subepicardial volume = 14.3%, subendocardial volume = 12.1%; capillary volume in systole, subepicardial volume = 9.1%, subendocardial volume = 8.9% (Table 2). If flow were continuous throughout the cardiac cycle and systole and diastole comprised equal portions of the cardiac cycle, then mean transit time (i.e., capillary volume/flow) would be ~0.89 seconds in subepicardium and 0.75 seconds in subendocardium. However, if subendocardial capillaries contained no red blood cells during systole, transit time would be reduced appreciably, and there is the possibility that O₂ off-loading would be impaired.

Capillary Diameter

The mean capillary diameter in “systolic” hearts (Table 2) was not significantly different from that previously found in the rat heart in diastole. It is possible that restricting measurements of capillary diameter to circular cross sections (<15% difference between smallest and largest diameter; see “Materials and Methods”) might bias the estimate of capillary diameter if reduction of capillary size occurred preferentially in one direction during fiber shortening. However, it is unlikely that this is the case. We have proven that capillaries are circular in diastolic hearts and have no indication that they are not circular in “systolic” hearts. Jv(c,f), estimated as Vv(c,f)/A(c), assuming circular cross-sectional profiles, was within 3.0% (subepicardium) and 5.5% (subendocardium) of that independently estimated via Equation 1.

There are at least two tenable hypotheses that might account for the lack of change in capillary diameter during “systole”: 1) Capillary-to-myocyte struts may provide external support for the capillary wall and prevent collapse (see above and References 42 and 44). 2) High tissue pressures act to collapse larger extracapillary vessels, thereby trapping blood in the capillaries. Because blood is incompressible, the intracapillary pressure would be expected to rise with tissue pressure during systole. This situation would clearly limit flow during systole as found by others. Also, it is consistent with previous reports that suggest that the larger microvascular units (i.e., >100 μm diameter) are preferentially collapsed during “systole.” In the present investigation, there was much evidence of erythrocytes trapped in the open lumen of capillaries. That these cells were not removed by saline flush before fixation indicates that, despite not being collapsed, these capillaries could not support flow.

Contrary to our findings, two previous reports have indicated that myocardial capillary diameter decreases during systole. However, it should be noted that in both studies measurements were restricted to the myocardial surface. It is possible that capillaries close to the myocardial surface behave differently compared with those deeper within the subepicardium and subendocardium. It is conceivable that the much lower tissue pressures at the myocardial surface would not occlude the extracapillary vessels, thereby permitting outflow from the capillary and allowing some reduction of capillary diameter.

That no change in capillary cross-sectional area occurs in “systole” is supported by the results of Judd and Levy, who report that, in vessels <100 μm in diameter, volume decreases by approximately the same amount (38%) in subepicardium and subendocardium in systole. This suggests that the volume decrease is not likely to result from compression per se (which is greater toward the subendocardium) but rather relates to reduced capillary length consequent to decreased fiber length, which is similar in subepicardium and subendocardium (Table 1).

The present investigation indicates that myocardial capillary volume is decreased at reduced fiber lengths and that this volume decrease is predominantly due to decreased capillary length rather than diameter. One expected consequence of this behavior is that the capillary endothelium may thicken in systole. It is possible that this mechanism may affect O₂ diffusion. However, the magnitude of this effect remains to be determined.

An interesting question arising from the relation between Jv(c,f) and sarcomere length (Figure 4) is whether capillaries in the heart are mechanically stretched at diastolic sarcomere lengths or, alternatively, whether they are actively shortened at “systolic” sarcomere lengths. Intravital microscopy studies of capillaries in the rat musculus extensor digitorum longus...
showed that individual capillaries stretch above sarcomere lengths $-2.1 \mu m$.\textsuperscript{14} When attempting to follow the characteristics of single capillaries from the morphometric analysis of populations of capillaries in perfusion-fixed tissue, several problems arise:\textsuperscript{14} 1) Not all capillaries may begin to stretch at the same sarcomere length. 2) Some capillaries may stretch to a greater or lesser extent than the muscle. 3) In fixed tissues, it is only possible to examine capillaries at one discrete sarcomere length. Thus, different muscles must be compared at different lengths. This unavoidably incorporates variability due to inherent differences between capillaries and/or muscles. However, morphometric data from 42 rat hind limb muscles are compatible with the notion that capillaries stretch above $-2.26 \mu m$ sarcomere length.\textsuperscript{14} Such investigations in the heart are complicated by the inability to gather data at long sarcomere lengths (i.e., 2.3–3.6 $\mu m$). It is apparent from Figure 4 that the variability of $J_v(c,f)$ between hearts precludes a formal analysis of the relation between $J_v(c,f)$ and sarcomere length. Irrespective of this, it is clear that $J_v(c,f)$ is reduced at sarcomere lengths. Also, the data are compatible with the notion that $J_v(c,f)$ remains constant or decreases to a lesser extent below 2.0–2.1 $\mu m$ sarcomere length (Figure 4).

**Implications for $O_2$ Diffusion**

A consequence of the higher $J_v(c,f)$ and $S_v(c,f)$ in diastole is that the potential for myocardial oxygenation is predicted to be lower in “systole.” In addition, the relative importance of changes in fiber versus capillary geometry on the structural capacity for oxygen delivery will depend on the site(s) of resistance to $O_2$ diffusion from capillary to fiber mitochondria. As for previous studies in skeletal muscle, measurements of myoglobin saturation in frozen cardiac myocytes indicate a uniformly low intracellular $P_{O_2}$, suggesting that the principal barrier to $O_2$ diffusion is found at the capillary-to-myocyte interface.\textsuperscript{30} This is in marked contrast to the Kroghian analysis, which considers intracellular $P_{O_2}$ to fall systematically with distance from the capillary. If the capillary-to-myocyte interface is indeed the principal barrier to $O_2$ diffusion between erythrocyte and mitochondrion, the maximum rate of $O_2$ diffusion into the myocyte will depend on the available area of the surface for diffusion (i.e., the capillary surface area), barrier thickness, and the transmural $O_2$ pressure gradient according to Fick’s law. Our data demonstrate that capillary length and surface area are significantly less in systole; therefore, for any given $O_2$ driving pressure, the capacity for myocyte oxygenation will be lower. Alternatively, if the resistance for $O_2$ diffusion lies within the fiber, increased $a(f)$ with systole will augment diffusion distances and increase the likelihood for intracellular hypoxia within those regions of the fiber most distant from the capillary. With respect to functional variables, it was beyond the scope of this investigation to determine the effect of systole on regional capillary flow patterns. However, the presence of open capillaries in subepicardium and subendocardium and erythrocytes in open capillaries suggests that flow is preserved in some capillaries and that even capillaries without flow retain their blood volume during systole.

This mechanism would act to increase mean transit time and may facilitate $O_2$ unloading.

**Conclusions**

The following conclusions are supported: 1) $a(f)$ increases and $Q_4(0)$ decreases with fiber shortening, as predicted from the reduction in sarcomere length at constant fiber volume. 2) In contrast to skeletal muscle, capillary tortuosity does not increase dramatically at sarcomere lengths $<2.0 \mu m$. Development of tortuosity may be constrained by the presence of capillary-to-myocyte collagenous struts\textsuperscript{41,42} or alternatively by the relatively high density of capillary branches. It is notable that pigeon pectoralis muscle, which has a dense network of capillary branches running perpendicular to the muscle fiber axis, also demonstrates little increase in capillary tortuosity with decreased sarcomere length.\textsuperscript{43} 3) Capillary length and surface area are decreased at short myocardial fiber lengths as a consequence of reduced $Q_4(0)$ accompanied by little change in capillary tortuosity. This implies that capillaries either compress longitudinally or recoil from diastole to systole. 4) Whether the predominant barrier for $O_2$ diffusion lies at the level of the capillary-to-myocyte interface or within the fiber, the geometrical potential for myocyte oxygenation will be lower at shorter sarcomere and fiber lengths (i.e., in systole).

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