Subendomyocardial Exhaustion of Blood Flow Reserve and Increased Fibrosis in Conscious Dogs With Heart Failure

Luc Hittinger, Richard P. Shannon, Sanford P. Bishop, Ricardo J. Gelpi, and Stephen F. Vatner

The effects of near-maximal coronary vasodilation were examined in conscious dogs with left ventricular (LV) failure after pressure overload hypertrophy induced by either aortic banding alone or aortic banding plus a peripheral arteriovenous shunt. The findings were compared with results in littermates with compensated LV hypertrophy and with a third group of normal dogs. At rest, there was a marked difference in the intramyocardial distribution of coronary flow, measured with radiolabeled microspheres. The endocardial/epicardial (endo/epi) flow ratio in the LV failure dogs was 0.96±0.08 as compared with control dogs (1.28±0.06, p<0.05) or dogs with compensated LV hypertrophy (1.23±0.08, p<0.05). During near-maximal coronary vasodilation with adenosine, all groups showed similar increases in subepimyocardial (epi) flow. While significant increases in subendomyocardial (endo) flow during adenosine infusion were seen in the control group (0.88±0.10 to 3.53±0.24 ml/min/g) and in dogs with compensated LV hypertrophy (1.12±0.14 to 3.60±0.16 ml/min/g), there was no change in endo flow in the LV failure dogs (1.55±0.20 to 1.71±0.47 ml/min/g) and a further significant reduction in the endo/epi flow ratio was observed (0.30±0.06, p<0.01). These hemodynamic changes were associated with chronic multifocal interstitial or discrete areas of fibrosis observed preferentially in endo layers. Thus, endo flow reserve is nearly exhausted in dogs with decompensated pressure overload LV hypertrophy, which may induce periodic episodes of endo ischemia resulting in myocyte necrosis and fibrosis, which in turn results in exacerbation of LV failure.

(Circulation Research 1989;65:971-980)

Alterations in the coronary circulation during the development of pressure overload left ventricular (LV) hypertrophy without LV failure have been studied extensively.1-8 The transmural distribution of blood flow at rest in compensated LV hypertrophy has been reported to be unchanged by most authors,2-8 while others have noted significant reductions compared with controls.9-10 However, maximal coronary flow reserve has been shown by most authors to be modestly impaired in compensated LV hypertrophy compared with control.3,7,9,11,12 The role that the impaired distribution of myocardial blood flow plays in the pathogenesis of LV failure remains to be established, in part due to a paucity of data regarding coronary blood flow regulation when pressure overload hypertrophy progresses to LV failure. Parrish et al13 noted that transmural myocardial blood flow actually increased at rest in four dogs with cardiac failure compared with those with a similar degree of compensated LV hypertrophy. However, the extent to which coronary vasodilator reserve is impaired in heart failure has not been established. Furthermore, although prior studies have speculated that the limitation of coronary flow reserve might lead to pathological alterations in the myocardium,9 evidence that structural changes are associated with the altered physiological response is lacking.

The initial goals of the present study were 1) examination of the alterations that occur in myocardial blood flow in the decompensated left ventricle following the development of pressure overload LV hypertrophy, 2) examination of the coronary vaso-
dilator reserve during near-maximal coronary vaso-
dilation induced by intravenous adenosine, and 3) 
determination of whether these alterations occur 
both subepicardially (epi) and subendocardially 
(endo) or selectively in endo layers. After initial 
experiments identified selective exhaustion of endo 
reserve in dogs with LV failure but only modest 
reductions in coronary reserve in dogs with com-
penstated LV hypertrophy, a fourth goal was deter-
mination of whether the alterations in myocardial 
blood flow regulation are associated with structural 
changes (e.g., fibrosis, which could be attributed to 
repeated episodes of endo ischemia) and whether 
these alterations in structure occur transmurally or 
selectively in endo layers.

Materials and Methods
Preparation of Model

Pressure overload LV hypertrophy was induced in 19 
dogs from three litters by implantation of a 
1-cm-wide Teflon band around the ascending aorta 
distal to the coronary arteries in mongrel puppies 
8–10 weeks of age. The band was implanted through 
a thoracotomy in the fourth right intercostal space 
by use of sterile surgical technique and sodium thiamyl anesthesia (12.5 mg/kg). The band was 
tightened until a thrill could be palpated over the 
aortic arch, and the chest was closed. A sham-
operated control was chosen at random from each 
litter. The puppies were allowed to recover from 
surgery and grow to maturation. The Teflon band 
created a fixed supravalvular aortic lesion, which 
became relatively more stenotic as the puppies 
grew. Six dogs spontaneously developed LV con-
gestive failure. Evidence of congestive failure 
included LV end-diastolic pressure (LVEDP) >20 
mm Hg, exercise intolerance, and evidence of pul-
monary congestion at autopsy. In four dogs with 
severe pressure overload LV hypertrophy but with-
out spontaneous heart failure (LVEDP >16 mm Hg), 
heart failure was induced by superimposition of a 
volume-overload lesion. After induction of general 
anesthesia with sodium thiamyl (8 mg/kg) and 
halothane (1 vol %), a 1.5-cm side-to-side bilateral 
iliac arteriovenous anastomosis was created to use 
of sterile surgical technique. The dogs were moni-
tored daily and allowed to recover for 1 week after 
this procedure. After a comparable period 
of aortic banding, the remaining nine banded dogs 
had no clinical signs of congestive heart failure, 
seven were subsequently studied during a state of 
compensated LV hypertrophy, while the other two 
were used for histologic studies.

Implantation of Instruments

Instruments were implanted in the aortic-banded 
dogs and in control dogs of similar weight at 13–14 
months of age. In aortic-banded dogs anesthesia was 
induced with sodium thiamylal (8 mg/kg i.v.) and 
maintained with halothane (1 vol %). In control dogs,
pentobarbital sodium (25 mg/kg) was used for anes-
thesia. An incision was made in the fifth left inter-
costal space by use of sterile surgical technique. 
Tygon catheters (Norton Plastics, Akron, Ohio) were 
implanted in the descending aorta, in the left atrium, 
and in the left ventricle. A miniature pressure gauge 
(Konigsberg Instruments, Pasadena, California) was 
also implanted in the left ventricle. The thoracotomy 
incision was closed in layers, and the animals were 
monitored daily and allowed to recover for 2 weeks 
before study. Seventeen aortic-banded dogs and 
eight control animals (including the three sham-
operated littermates) were instrumented in this fash-
ion. Animals used in this study were monitored in 
accordance with the guidelines of the Committee on 
Animals of Harvard Medical School and those pre-
pared by the Committee on Care and Use of Labo-
atory Animals of the Institute of Laboratory Ani-
mal Resources, National Council (DHEW 
publification No. (NIH) 85-23, revised 1985).

Experimental Measurements

Statham P23 ID strain gauge manometers (Gould, 
Cleveland, Ohio) were calibrated against a mercury 
manometer. LV pressure was measured by use of the 
solid-state miniature pressure gauge calibrated in 
vitro with a mercury manometer, and in vivo by 
use of the LV pressure measurements from the 
catheters and Statham strain gauge manometers. The 
data were recorded on a multichannel oscillograph 
(Gould-Brush, Cleveland, Ohio) and multichannel 
tape recorder (Honeywell, Denver, Colorado).

Regional myocardial blood flow was measured 
with isotopically labeled microspheres (15±2 μm 
diameter, New England Nuclear, Boston, Massa-
chusetts) in eight control dogs, seven dogs with 
compensated LV hypertrophy, and 10 dogs with 
LV failure. The radioactive label of the micro-
spheres (141Ce, 114In, 51Cr, 193Ru, 99Nb, 85Sr, or 46Sc) 
was chosen randomly. The microspheres were sus-
pended in 0.01% Tween 80 solution (10% dextran) 
agitated by direct application of an ultrasonic probe 
to ensure dispersion of the microspheres and placed 
in an ultrasonic bath for at least 30 minutes before 
junction. Before the microspheres were injected 
into the animals, 0.7 ml of Tween 80 dextran 
solution (without microspheres) was injected to 
determine if the diluent for the microsphere suspension 
would have an adverse effect on measurement of 
cardiac or systemic hemodynamics. Then 1 mil-
lion to 2 million microspheres, suspended in the 10% 
dextran, were injected through the catheter 
implanted in the left atrium for determination of 
blood flow. A reference sample of arterial blood 
was withdrawn (7.5 ml/min) from a catheter inserted 
into the descending aorta. Reference sample 
withdrawal was initiated 15 seconds before microsphere 
junction and continued for approximately 90 sec-
onds after the injection was completed (total with-
drawal time was 120 seconds).
Experimental Protocol

Experiments were performed in a quiet, darkened laboratory with the unanesthetized conscious dog resting comfortably on its right side. The near-maximal coronary flow reserve of eight control dogs, seven dogs with compensated LV hypertrophy, and 10 dogs with LV heart failure was assessed by the intravenous administration of adenosine (4.7 μM/kg/min). After control measurements were made of aortic pressure, LV systolic and end-diastolic pressures, and heart rate, radioactive microspheres were injected for measurement of regional myocardial blood flow. Near-maximal coronary vasodilation was then achieved by the intravenous infusion of adenosine. After all the parameters measured had achieved a new steady-state level (approximately 5 minutes of adenosine infusion), radioactive microspheres again were injected for measurement of regional myocardial blood flow during maximal coronary vasodilation.

Postmortem Evaluation

Heart tissue from some dogs used in the study of coronary flow reserve was used for other studies requiring fresh tissue and, thus, was not available for pathological study. In two dogs with compensated LV hypertrophy and two dogs with LV failure, the heart remaining after removal of tissues was immersion fixed in 10% phosphate-buffered formalin. In five control dogs, three dogs with compensated LV hypertrophy, and three dogs with LV failure, the hearts were perfusion fixed with glutaraldehyde. After the animals were anesthetized with sodium pentobarbital (30 mg/kg), a lethal dose of KCl was given, and the heart was perfused through the ascending aorta at 100 mm Hg with 2% phosphate-buffered glutaraldehyde. After the animals were anesthetized with sodium pentobarbital (30 mg/kg), a lethal dose of KCl was given, and the heart was perfused through the ascending aorta at 100 mm Hg with 2% phosphate-buffered glutaraldehyde after blood was washed from the coronary circulation with saline. The atria and right ventricular free wall were removed from the left ventricle and septum. Major epicardial blood vessels and valves were removed, and the septum was separated from the LV free wall. The right ventricular free wall and LV free wall plus septum were weighed separately.

Histologic study was conducted on five control dogs, five dogs with compensated LV hypertrophy (LV end-diastolic pressure less than 12 mm Hg), and five dogs with LV failure (four spontaneous and one with LV end-diastolic pressure of 18 mm Hg preshunt and 23 mm Hg postarteriovenous shunt). Transmural sections of myocardium in both horizontal and vertical planes were taken for histologic examination from the right ventricular free wall, the interventricular septum, and the posterior LV wall, anterior LV wall, and LV free wall. A total of eight sections were taken from the left ventricle and interventricular septum and two from the right ventricle. Care was taken to avoid areas adjacent to implanted instrumentation. Tissues were embedded in paraffin and 5-μm sections were stained with hematoxylin and eosin, Gomori's aldehyde fuchsin trichrome, and picrosirius red.

The stained sections were marked for identification of epi, mid, and endo thirds of the ventricular wall. Tissues were analyzed quantitatively for determination of the type and amount of connective tissue response. The quantitative approach used a video imaging system (Image Technology, Deer Park, New York) in which a microscope with video camera is interfaced to an IBM XT computer with software programmed to calculate the percentage of pixels exceeding a selected density. Picrosirius red-stained sections were examined with Nomarski optics adjusted to allow low transmission through red-stained connective tissue relative to yellow muscle tissue. Three sections from each heart (anterior wall, posterior wall, free wall) were evaluated with a minimum of 30 fields measured for endo thirds and 20 fields for midwall and epi thirds. Fields were measured by use of the x10 objective with the limiting screen on the video set for a 600×600-μm area. Adjacent fields were measured following an alternating inner-to-outer, outer-to-inner pattern through each region, excluding fields with technical artifacts or large vascular space. Mean volume percent connective tissue for each transmural region of each animal was calculated from the three slides, and these values were combined for determination of group means.

Blood Flow and Regional Resistance Calculation

Samples of myocardial tissue from the LV free walls were subdivided into transmural layers from epi to endo, weighed, and placed in a gamma counter (Canberra Industries, Meriden, Connecticut) with appropriately selected energy windows. The raw counts were corrected for background and crossover and compared with the reference blood sample to obtain flow expressed in milliliters per minute per gram of tissue. Endo/epi blood flow ratios were obtained by dividing blood flow of the endo layer by that of the epi layer. In the control dogs, mean aortic pressure was used for assessment of coronary perfusion pressure. In the dogs with compensated LV hypertrophy and LV failure, mean coronary perfusion pressure was computed according to the technique described by Bache et al., which uses the LV pressure averaged by planimetry from the beginning of the upstroke of the aortic pressure to the dicrotic notch as systolic pressure and the aortic pressure averaged from the dicrotic notch to the beginning of the next systolic upstroke as diastolic pressure. Mean, endo, and epi coronary resistances per gram were calculated as the quotient of mean coronary perfusion pressure and mean, endo, and epi blood flows, respectively.

Data Analysis

Statistical analysis was performed using a statistical computing package (Statistical Program for the Social Sciences [SPSS], Chicago, Illinois) on an
IBM AT computer. The data are reported as mean±SEM. The effects of adenosine infusion on the measured variables were assessed for statistical significance by Student's *t* test for paired comparisons. Differences in the measured variables between the control group, LV hypertrophy group, and LV failure group were assessed for statistical significance by analysis of variance, with group differences analyzed by unpaired *t* test and use of a Bonferroni correction.15

**Results**

**Morphology**

Table 1 reveals that the LV free wall plus septum/body weight ratio was significantly greater both in dogs with compensated LV hypertrophy and dogs with LV failure compared with control dogs. There was no significant difference between the LV free wall plus septum weight or the LV/body weight ratio in dogs with compensated LV hypertrophy and those with LV failure. There was also no difference between these two groups in the time from aortic banding to postmortem measurement. There was no difference in right ventricular weight-to-body weight ratio among the three groups.

**Hemodynamic Effects of LV Failure**

At baseline, the LV systolic pressure was significantly greater both in dogs with compensated LV hypertrophy and dogs with LV failure compared with control dogs (Table 2). The LV end-diastolic pressure was significantly greater in the LV failure dogs compared with either dogs with compensated LV hypertrophy or controls. Resting heart rates were similarly greater in dogs with LV failure compared with the other two groups. The peak-to-peak LV-to-aortic systolic pressure gradient was similar in dogs with LV failure (152±11 mm Hg) and dogs with compensated LV hypertrophy (148±23 mm Hg). Mean arterial pressure was less in the dogs with LV failure compared with the other two groups, but the difference was insignificant. At baseline, there was no significant difference in endo and epi blood flow between control dogs and dogs with compensated LV hypertrophy (Table 3). However, both endo and epi blood flows were

<table>
<thead>
<tr>
<th>LV systolic pressure (mm Hg)</th>
<th>Control</th>
<th>LV hypertrophy</th>
<th>LV failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125±3</td>
<td>218±9†</td>
<td>237±18†</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>218±9†</td>
<td>203±15†</td>
<td>224±21†</td>
</tr>
<tr>
<td>LV failure</td>
<td>237±18†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LV end-diastolic pressure (mm Hg)</th>
<th>Control</th>
<th>LV hypertrophy</th>
<th>LV failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.6±1.0</td>
<td>12.6±1.5†</td>
<td>26.5±2.8†</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>12.6±1.5†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV failure</td>
<td>26.5±2.8†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>Control</th>
<th>LV hypertrophy</th>
<th>LV failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89±5</td>
<td>99±5</td>
<td>142±6†</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>99±5</td>
<td>126±7*</td>
<td>137±7</td>
</tr>
<tr>
<td>LV failure</td>
<td>142±6†</td>
<td>128±7*</td>
<td>137±7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean arterial pressure (mm Hg)</th>
<th>Control</th>
<th>LV hypertrophy</th>
<th>LV failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97±3</td>
<td>99±5</td>
<td>85±6</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>99±5</td>
<td>65±3*</td>
<td></td>
</tr>
<tr>
<td>LV failure</td>
<td>85±6</td>
<td>59±7*</td>
<td></td>
</tr>
</tbody>
</table>

LV, left ventricle.

*p<0.05 vs. baseline.

†p<0.05 vs. control.

---

**Table 1. Postmortem Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LV hypertrophy</th>
<th>LV failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (kg)</td>
<td>29±2</td>
<td>28±2</td>
<td>24±1</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free wall + septum wt (g)</td>
<td>129±11</td>
<td>192±13§</td>
<td>189±9§</td>
</tr>
<tr>
<td>Free wall + septum wt/body wt (g/kg)</td>
<td>4.5±0.3</td>
<td>7.0±0.4§</td>
<td>7.9±0.6§</td>
</tr>
<tr>
<td>Period banded (months)</td>
<td>13±2</td>
<td>12±1</td>
<td></td>
</tr>
<tr>
<td>Right ventricular wt (g)</td>
<td>49±3</td>
<td>45±6</td>
<td>46±3</td>
</tr>
<tr>
<td>Right ventricular wt/body wt (g/kg)</td>
<td>1.7±0.1</td>
<td>1.6±0.2§</td>
<td>1.9±0.1</td>
</tr>
</tbody>
</table>

LV, left ventricle.

*Includes eight dogs studied for coronary flow and four additional dogs studied for histology.

†Includes seven dogs studied for coronary flow and two additional dogs studied for histology.

§Includes 10 dogs studied for coronary flow; no dogs were studied for histology.

*p<0.01 vs. control. There were no significant differences between LV hypertrophy and LV failure groups.

---

**Table 2. Hemodynamic Response to Adenosine**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV systolic pressure (mm Hg)</td>
<td>8</td>
<td>125±3</td>
<td>113±6*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>218±9†</td>
<td>203±15†</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>10</td>
<td>237±18†</td>
<td>224±21†</td>
</tr>
<tr>
<td>LV end-diastolic pressure (mm Hg)</td>
<td>8</td>
<td>8.6±1.0</td>
<td>5.7±1.4*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>12.6±1.5†</td>
<td>13.2±1.8†</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>10</td>
<td>26.5±2.8†</td>
<td>20.2±2.5†</td>
</tr>
<tr>
<td>LV failure</td>
<td>8</td>
<td>89±5</td>
<td>126±7*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>7</td>
<td>99±5</td>
<td>128±7*</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>10</td>
<td>142±6†</td>
<td>137±7</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>8</td>
<td>97±3</td>
<td>73±5*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>99±5</td>
<td>65±3*</td>
</tr>
<tr>
<td>LV hypertrophy</td>
<td>10</td>
<td>85±6</td>
<td>59±7*</td>
</tr>
</tbody>
</table>

LV, left ventricular.
significantly greater in dogs with LV failure at rest compared with the control dogs. The mean endo/epi flow ratio was significantly less in LV failure dogs compared with either dogs with compensated LV hypertrophy or control dogs. There was no difference in the endo/epi flow ratio between six dogs with spontaneous LV failure (0.91 ± 0.10) and four with LV failure after arteriovenous shunts were superimposed on severe LV hypertrophy (1.05 ± 0.11). Using the measurement of regional myocardial blood flow and coronary perfusion pressure determined as noted previously, an index of mean regional coronary resistance was calculated for the endo and epi regions (Table 3). At baseline, coronary vascular resistance was significantly lower only in the epi regions of the dogs with LV failure as compared with control dogs, although there was a trend toward lower coronary vascular resistance in both regions in both the LV hypertrophy and LV failure groups.

**Effects of Adenosine (Tables 2 and 3)**

In control dogs, dogs with LV hypertrophy, and dogs with LV failure, adenosine decreased mean arterial pressure by 24±5 mm Hg (p<0.01), 34±5 mm Hg, (p<0.01), and 26±4 mm Hg (p<0.01), respectively (Table 2). LV systolic pressure decreased in all groups during adenosine infusion, but the decrease was statistically significant only in the control dogs (12±5 mm Hg, p<0.03). LV end-diastolic pressure fell in the control dogs (2.9±1 mm Hg, p<0.04) but did not change in the LV hypertrophy group and tended to increase during adenosine infusion in the LV failure group, raising the possibility of impairment in LV function. Heart rate increased significantly in control dogs (37±10 beats/min, p<0.01) and dogs with LV hypertrophy (29±10 beats/min, p<0.03) in response to adenosine but did not change in dogs with LV failure.

The effects of adenosine on myocardial blood flow are shown in Table 3. The epi blood flow increased significantly (p<0.01) in all three groups. However, the significant increase in the endo blood flow seen in the control dogs and dogs with compensated LV hypertrophy was not evident in the LV failure group. During near-maximal coronary vasodilation, the endo/epi perfusion ratio fell significantly both in control dogs (from 1.28±0.06 to 0.71±0.05, p<0.01) and dogs with LV hypertrophy (from 1.23±0.08 to 0.62±0.10, p<0.01). However, in the LV failure group, the endo/epi ratio fell to a significantly lower level (from 0.96±0.08 to 0.30±0.06, p<0.01), further reflecting the profound inability of the endo to augment flow during maximal coronary vasodilation.

After near-maximal coronary vasodilation with adenosine, epi resistance fell significantly in all three groups to similar minimal levels. Notably, during adenosine infusion there was no significant decrease in endo resistance in the LV failure dogs (89±15 vs. 97±16 mm Hg/ml/min/g for controls), while in both controls and dogs with compensated LV hypertrophy there was a significant fall in endo resistance (Figure 1). However, while the minimum level of endo resistance was elevated in dogs with LV hypertrophy compared with control dogs, it was
Matory cells were absent. There were focal areas of loose areolar connective tissue elements, and inflammation stained with trichrome, there was no evidence of collagen fibrils were closely arranged and darkly to have been present for longer than 1-2 months: duration). The multifocal areas of fibrosis appeared some of the implanted instruments (1-4 weeks' recent necrosis other than that associated with myocardial fibrosis attained significant reductions. LV, left ventricular.

not elevated to the same extent as was observed in dogs with LV failure.

Myocardial Fibrosis

Heart weight and functional characteristics of the additional dogs used for histology (four controls, two with compensated LV hypertrophy) were not different from those used for the blood flow analysis.

Visual examination of the surface of the multiple cut slices of myocardium failed to reveal evidence of fibrosis in any animals, other than that found in the immediate vicinity of implanted instruments. Histologic examination of the tissue from the LV failure group revealed multifocal areas of dense or diffuse interstitial fibrosis, mainly in the endo regions, and an increase in perivascular connective tissue. Tissue between these microscopic focal lesions had no detectable increase in connective tissue by light microscopy. The control dogs had no detectable fibrosis beyond the normal occasional strands of connective tissue between muscle bundles and in perivascular regions. Occasional foci of fibrosis were found in the LV hypertrophy group but, subjectively, appeared much less common than in the LV failure group. Figure 2 illustrates representative photomicrographs of the LV endo in a control dog and in three dogs with LV failure that exhibited focal reparative fibrosis with myofiber replacement, interstitial fibrosis, and perivascular fibrosis, respectively. There was no evidence of recent necrosis other than that associated with some of the implanted instruments (1-4 weeks' duration). The multifocal areas of fibrosis appeared to have been present for longer than 1-2 months: Collagen fibrils were closely arranged and darkly stained with trichrome, there was no evidence of loose areolar connective tissue elements, and inflammatory cells were absent. There were focal areas of confluent connective tissue scar ranging in diameter from 0.1 to 0.3 mm in two dogs with compensated hypertrophy and in all dogs with LV failure, and two dogs with LV failure had single foci up to 8 mm in diameter. These focal areas of fibrosis were interpreted to represent healed areas of myocardial necrosis. The size of the interstitial areas of fibrosis seldom exceeded 2 mm.

Figure 3 depicts the quantitative evaluation using the video system. Compared with control dogs, the LV failure group exhibited increased mean volume percent connective tissue content, particularly in the endo region but also in the midwall. There was a trend toward increased connective tissue in the endo and midwall of the dogs with LV hypertrophy, but the changes were not statistically significant. Thus, dogs with LV failure had a predilection for multifocal fibrosis in the endo relative to the epi (p<0.01), suggesting that the myocardial fibrosis, like the impairment in coronary flow reserve, was preferential for the inner myocardium. The one dog with an arteriovenous shunt in addition to aortic banding exhibited extensive endo fibrosis. However, before creation of the shunt this dog had an LV end-diastolic pressure that was higher than that of the other dogs with compensated LV hypertrophy and closer to the minimum level of the dogs in LV failure.

Discussion

In the present investigation, we examined changes in myocardial blood flow in conscious dogs with compensated LV hypertrophy and dogs with LV hypertrophy and failure induced by aortic banding in puppies. All animals were studied in the awake state for elimination of the complicating influences of anesthesia and recent surgery. While dogs with compensated LV hypertrophy and dogs with LV failure were banded for similar durations and exhibited similar amounts of hypertrophy, the dogs with LV failure were characterized by markedly elevated LV end-diastolic pressure, exercise intolerance, and evidence of pulmonary congestion at autopsy. Thus, the development of LV failure was not due simply to duration of the lesion or severity of the LV hypertrophy. The major findings in dogs with LV failure included 1) significant alterations in transmural distribution of blood flow at rest, as evidenced by endo hypoperfusion; 2) exhaustion of endo coronary flow reserve during near-maximal vasodilation; and 3) multifocal areas of fibrosis preferentially observed in endo regions of hypoperfusion and reduced flow reserve.

Alterations in transmural distribution of blood flow have been studied extensively in compensated pressure overload hypertrophy produced by aortic banding. In most studies, no significant differences have been found in the endo/epi flow ratio at rest between control and compensated LV hypertrophy in either anesthetized3,11 or conscious animal preparations.4-8,16,17 Our findings are consistent with
**FIGURE 2.** Photomicrographs of LV endocardium of a control dog (panel A) and three dogs with LV hypertrophy and failure (panels B, C, and D). Tissues were perfusion fixed with glutaraldehyde, resulting in distention of blood vessels. No fibrosis is present in panel A. Focal fibrosis with myofiber replacement is present in panel B, interstitial fibrosis in panel C, and perivascular fibrosis in panel D. Magnification bar in panels A, B, and D equals 100 μm, Gomori's aldehyde fuchsin stain, paraffin embedded; magnification bar in panel C equals 50 μm, toluidine blue stain, methacrylate embedded.
this extensive literature. Rembert et al\(^9\) found a significant reduction in endo/epi flow ratio at rest in dogs with severe LV hypertrophy induced by surgical coarctation of the ascending aorta; however, the endo maintained a flow reserve capacity in response to adenosine and reactive hyperemia. Bache’s laboratory has observed significant decreases in endo/epi flow ratio at rest in some instances\(^5,17\) but not in others.\(^4,8,17\) However, in most studies of LV hypertrophy without failure in conscious dogs, the endo/epi flow ratio remained above unity, as was observed in the current investigation.

In the present study, myocardial blood flow was higher in the LV failure dogs compared with control dogs or dogs with compensated LV hypertrophy. This finding likely relates to the increased myocardial oxygen demand associated with the higher heart rate and greater wall stress in the LV failure group. However, at rest, the endo/epi ratio was below unity in the LV failure dogs (0.96±0.08) and significantly less (p<0.05) than that observed in the control dogs or dogs with compensated LV hypertrophy, suggesting greater alterations in transmural distribution of flow at baseline in LV failure. Our findings are consistent with those of Parrish et al,\(^13\) who also observed higher baseline myocardial blood flow in four dogs with LV failure as well as a decrease in LV endo/epi flow ratio below unity (0.96±0.11).

Alterations of transmural blood flow, as manifested by decreases in endo/epi flow ratio, have been reported in compensated LV hypertrophy in response to rapid pacing at rates of 250 beats/min,\(^4,7,14,17,18\) exercise,\(^5,8\) and adenosine.\(^3,6,7,11,16\) While a modest decrease in coronary flow reserve in response to adenosine was observed in dogs with compensated LV hypertrophy in the present study, there are several important differences between the results in compensated LV hypertrophy and in LV failure: 1) The decrease in endo/epi flow ratio to 0.30±0.06 in LV failure was more striking than the ratio reported previously in response to adenosine in compensated LV hypertrophy as well as the ratio in the LV hypertrophy dogs reported here; 2) coronary flow reserve and maximum vasodilator capacity were exhausted in the endo while coronary flow reserve was not impaired and vasodilator capacity was reduced only modestly in the epi; 3) the exhaustion in coronary flow reserve in response to adenosine was associated with a predilection for endo reparative fibrosis. The differences between the groups could not be ascribed to either the duration of aortic banding or the severity of the hypertrophy, since these factors were similar in the two groups. Furthermore, the pronounced fall in endo/epi flow ratio with adenosine in the dogs with LV failure could be attributed neither to reduced coronary perfusion, since that variable was elevated, nor to tachycardia, since heart rates were almost identical in both groups during adenosine infusion. Thus, in LV failure at rest, the endocardium was operating at maximal coronary reserve. These data conflict with the observations of Parrish et al,\(^13\) who reported no significant impairment in transmural myocardial blood flow after adenosine in a single dog with LV failure. The difference is likely reconciled by the greater number of animals reported here and the more severe degree of LV failure, as manifested by higher LV end-diastolic pressure at rest in our dogs.

The increase in endo connective tissue in the dogs with LV failure in the present study lends morphological evidence that there was underperfusion of the inner myocardium, presumably during periods of stress. Although a causal relation cannot be demonstrated from these findings, if there were repeated bouts of transient endo ischemia, as suggested by the severe impairment in endo flow reserve, a reasonable consequence would be the repair of damaged endo foci with fibrous connective tissue. Previous studies have shown that age, species, and the type, duration, and severity of pressure overload lesion are all factors influencing the connective tissue content in hypertrophied myocardium. However, duration of the aortic banding lesion and severity of LV hypertrophy were not factors in the present investigation, since they were similar in the dogs with compensated and decompenated LV hypertrophy. Recently, Weber et al\(^19\) reported dynamic changes in collagen type and content in a primate model with pressure overload hypertrophy induced by perinephritic hypertension. Myocyte necrosis and reparative fibrosis were noted after 88 weeks, but the selectivity of the lesion to the endo and its relation to changes in coronary flow were not studied. Rembert et al\(^9\) found minimal fibrosis selective to the endo but concluded that it was not related to the alterations in coronary blood flow and may have been secondary to chronic instrumentation. In contrast, the present study demonstrated the fibrotic process to be located primarily in the endo in dogs with pressure overload hypertrophy and failure induced by aortic banding,
even in noninstrumented animals, and correlated this finding with severe impairment in endo flow.

If the fibrosis was a consequence of either the experimental banding procedure used to produce LV hypertrophy or the elevated coronary perfusion pressure, then it should have been observed diffusely in all layers, as was noted by Bishop and Melsen in cats undergoing pulmonary artery banding and rabbits with aortic banding. The model used in this study utilized the growth of the animal to induce a gradually increasing degree of stenosis and thus avoided the complications produced by sudden pressure overload. In the present study, the fact that fibrosis was found preferentially in the endo suggests that the increase in connective tissue content was a consequence of the endo hypoperfusion in the dogs with LV failure and not a consequence of either the LV hypertrophy alone or the elevated coronary perfusion pressure. It is also reasonable to consider the possibility that the increased connective tissue was the cause of the endo hypoperfusion and the LV failure. However, prior studies using similar models of pressure overload LV hypertrophy, but without failure, have not reported increased connective tissue in the endo, suggesting that this finding is a consequence of the endo ischemia and plays a role in the pathogenesis of LV decompensation. Our data demonstrate a trend toward an increase in endo fibrosis even in dogs with compensated LV hypertrophy; however, this finding was not statistically significant and was not near the magnitude observed in dogs with aortic banding of similar duration but in which LV failure had developed. It is interesting that a study in human hearts also observed fibrosis in LV hypertrophy and a greater amount of fibrosis in LV failure. It is also important to recognize the possibility that these findings of preferential endo fibrosis and reduced endo coronary reserve are specific to pressure overload models. In rats with volume overload, others have observed no increase in fibrosis. In the current investigation, the one dog with volume overload superimposed on severe pressure overload hypertrophy had a degree of endo fibrosis similar to that of dogs with spontaneous LV failure due to pressure overload alone.

In summary, the lack of significant increase in endo blood flow when challenged with a potent vasodilator indicates that the endo at rest is operating at or near the limits of coronary flow reserve in dogs with this model of LV failure and is highly vulnerable to ischemia. This impairment in endo flow reserve was more severe than has been previously observed or was noted in the present study in compensated pressure overload hypertrophy. In contrast, in the epi, blood flow tended to rise even higher in response to adenosine in dogs with compensated LV hypertrophy and dogs with LV failure, although the minimal level of epi coronary resistance was elevated modestly in the dogs with LV failure. The fact that the selective impairment in endo flow was associated with enhanced fibrosis found preferentially in the endo layers suggests that this impairment in flow reserve is sufficiently severe to cause periodic episodes of ischemia and should be considered as one potential mechanism in the pathogenesis of decompensation from LV hypertrophy to LV failure. It must also be considered that the fibrosis, in turn, leads to alterations in compliance and further endo ischemia, thereby exacerbating the decompensation process.

References


**KEY WORDS** • heart failure • myocardial fibrosis • coronary reserve • coronary blood flow • hypertrophy • adenosine
Subendomyocardial exhaustion of blood flow reserve and increased fibrosis in conscious dogs with heart failure.

L Hittinger, R P Shannon, S P Bishop, R J Gelpi and S F Vatner

_Circ Res._ 1989;65:971-980
doi: 10.1161/01.RES.65.4.971

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/65/4/971

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/