Reduced Volume Fraction of Myofibrils in Myocardium of Patients with Decompensated Pressure Overload

FRANZ SCHWARZ, M.D., JUTTA SCHAPER, M.D., DIETER KITTSTEIN, M.D., WILLEM FLAMENG, M.D., PAUL WALTER, M.D., AND WOLFGANG SCHAPER, M.D.

SUMMARY The relation between quantitative ultrastructural changes of the left ventricular (LV) myocardium and contractile function was studied in patients with chronic aortic stenosis (AS). The volume fractions of myofibrils, sarcoplasm and mitochondria in myocardial cells were determined by electron microscopic morphometry in small LV tissue samples of 19 patients with AS. Interstitial fibrosis was measured by light microscopic morphometry. Transmural biopsies of the LV free wall perfused by the anterior descending branch of the left anterior descending coronary artery (LAD) were obtained during aortic valve replacement. LV function was analyzed from preoperative right- and left-heart catheterization and angiography. Group 1 consisted of seven patients with ejection fractions (EFs) greater than 55% and mean left atrial pressure (LAP) less than 15 mm Hg. Group 2 consisted of 12 patients with EFs less than 55% and mean LAP greater than 15 mm Hg. Patients in group 1 had lower LV end-diastolic volume (91.9 vs 145.3 ml/m², p < 0.05) and lower LV muscle mass (148.3 vs 199.8 g/m², p < 0.05) than patients in group 2. The volume fraction of myofibrils was higher in group 1 than in group 2 (48.4 vs 42.1%, p < 0.05), while volume fractions of sarcoplasm (31.7 vs 36.0%) and mitochondria (20.9 vs 22.0%) were comparable (p > 0.05). Interstitial myocardial fibrosis did not differ between groups (16.3 vs 14.7%, p > 0.05). Biopsies from the area perfused by the LAD in 10 additional surgical patients who had coronary artery disease with moderate LAD stenosis and normal wall motion in the area of LV free wall perfused by the LAD were taken as controls for morphometric data. No significant difference of ultrastructural data was found between group 1 and controls. The volume fraction of myofibrils was lower in group 2 than in controls (42.1 vs 52.9%, p < 0.001), and the volume fraction of sarcoplasm was higher (36.0 vs 21.1%, p < 0.001). Mitochondria and interstitial fibrosis did not differ in group 2 and controls (p > 0.05).

Thus, intracellular reduction in the volume fraction of myofibrils was the major morphologic finding in LV biopsy samples of patients with decompensated pressure overload.

ULTRASTRUCTURAL degenerative changes of the myocardium in hypertrophied right and left ventricles have been proposed as morphologic correlates of impaired cardiac function. However, no attempt has been undertaken to quantify these changes by morphometry of myocardial biopsy samples. Therefore, we examined the ultrastructure of transmural myocardial biopsy tissue from patients with aortic stenosis (AS) and quantitated the intracellular volume fractions of myofibrils (contractile material), sarcoplasm and mitochondria by electron microscopic morphometry, and the interstitial myocardial fibrosis by light microscopic morphometry. We then correlated quantitative ultrastructural findings with preoperative hemodynamics in these patients.

Methods

Patients

We studied 19 surgical patients, 15 men and four women, mean age 47 ± 10 years (± sd) (range 34–66 years), who had chronic AS. All patients underwent right- and left-heart catheterization, including left ventricular (LV) angiography and selective coronary arteriography, within 2 months before operation because of angina or dyspnea on exertion (n = 15) or severe congestive symptoms (n = 4). No patient had an acute event between catheterization and operation. Ten additional patients, ages 44–61 years, who had coronary artery disease and underwent LV catheterization and angiography and selective coronary arteriography within 2 months before coronary bypass surgery were also studied. Coronary surgery was done because of chronic angina in all 10 patients. Seven patients received two grafts and three patients received three grafts. The degree of the stenosis of the left anterior descending coronary artery (LAD) was measured using the technique of Brown et al., in which the cross section of the stenosis and the pre- and poststenotic vessel segments from two perpendicular angiographic views chosen at end-diastole are measured. We have used this technique and found good reproducibility of the method. The LAD stenosis was mild to moderate (40–85% luminal reduction) and averaged 71.5 ± 13.9%. No patient had electrocardiographic signs of transmural or subendocardial anterior infarction. LAD wall motion was measured by hemicaxis shortening, as previously described, and was 43.7 ± 8.3%. Our normal value for LAD wall motion is 39.4 ± 10.3% (n = 22). Because LAD wall motion in coronary patients was not significantly different from that in normal subjects (p > 0.05), we
considered regional function in the LAD perfusion area to be normal in the coronary group. Myocardial biopsies were obtained in the coronary group from the center of the LAD perfusion area. The 10 patients with coronary artery disease were selected from a larger series of patients studied for evaluation of myocardial ultrastructure in coronary artery disease.

Catheterization Study

All patients were studied during normal sinus rhythm without premedication. Right ventricular catheterization was done using a Brockenbrough catheter. Left-heart catheterization was performed using the transseptal approach; the aorta was catheterized using the transfemoral retrograde route. Pressures were recorded on an Oscillograph direct-writing system with Statham P23Db transducers before injection of contrast material. LV end-diastolic pressure was measured after the “a” wave. Single-plane, 35-mm cineangiograms of the left ventricle were filmed at 48 frames/sec in the 30° right anterior oblique projection after injection of 50 ml of Urografin 76. During LV injection through the Brockenbrough catheter in patients with AS, no mitral regurgitation was seen in normally conducted sinus beats. The aortic pressure pulse and the ECG were recorded simultaneously at a paper speed of 100 mm/sec. We performed aortic root angiography in all patients to estimate the degree of aortic regurgitation. Selective coronary arteriography was done using the Judkins technique. LV volumes and ejection fraction (EFs) were determined by the area-length method. Correction factors for magnification and pin-cushion distortion were obtained. The earliest well-opacified cardiac cycle was chosen for analysis, excluding extrasystolic and postextrasystolic beats. Calculated volumes were corrected to true volumes. The mean normalized systolic ejection rate was calculated as suggested by Peterson et al. End-diastolic thickness of the LV free wall was measured in the right anterior oblique projection and LV muscle mass was determined according to the method of Rackley et al. with the modification of Trenough et al. Peak systolic wall stress (at the equator) was calculated using the formula of Falsetti et al. and the method of Gaasch et al.

Surgery

All patients were operated with the use of total cardiopulmonary bypass and a disposable bubble-type oxygenator. A transmural needle biopsy specimen measuring 1.5 mm in diameter (Tru-Cut biopsy needle, Travenol Laboratories) was obtained from the center of the area perfused by the LAD. The biopsy was taken from the heart before cross clamping of the aorta. Immediately after biopsy, the tissue was fixed in cacodylate-buffered 6% glutaraldehyde. The osmolality of the cacodylate buffer was 190 mosmol/l without the glutaraldehyde and 290 mosmol/l with the added glutaraldehyde. After immersion fixation for 24 hours at 4°C, the samples were rinsed in 0.1 M cacodylate buffer with 7.5% sucrose for 12 hours at 4°C. After postfixation for 1 hour in 2% osmium tetroxide veronal acetate buffer at pH 7.4 with 4% sucrose at 4°C, the tissues were dehydrated in graded series of ethanol, treated with propylene oxide and embedded in epon-812.

Electron Microscopic Morphometry

Thin sections (0.5 µm) for electron microscopic examination were cut with a microtome (LKB Ultratome III), mounted on uncoated copper grids, stained with saturated aqueous uranyl acetate and lead citrate and evaluated with an electron microscope (Philips EM 300). Sections were put on a grid and localized at low-power magnification under the electron microscope. Then, at a magnification of x 10,000, electron micrographs were made using a random sampling procedure. In each square of the grid, two electron micrographs were made using the upper left and the lower right corners as orientation for the selection process. A counting grid with 144 intersections (points) was then put over the photographs. According to the principles of morphometry, counting the number of points overlying a certain structure results in a quantitative determination of the volume of the structure under investigation in relation to the volume of the entire tissue. The volume fractions of three intracellular compartments — myofibrils, sarcoplasm and mitochondria — were determined. The nucleus was excluded from the counting process. The percentage of each compartment was obtained by dividing the number of points for each compartment by the total number of points counted. A transmural sample from each patient was studied, and 12–50 micrographs (average 29 micrographs) were prepared from each sample, for a total of 841 electron micrographs. For each micrograph, 144 points were counted (i.e., a total of 121,104 points). Variability of the method was tested by the following procedure. Electron micrographs from nine patients were prepared at magnifications of x 5000, x 10,000 and x 16,000. For each tissue and magnification, at least 24 micrographs were prepared and counted. The results were compared by linear regression analysis for the different magnifications, and the correlation coefficients were 0.94, 0.97, and 0.98.

Light Microscopic Morphometry

Semithin sections (1–2 µm thick) were prepared and stained with alkaline toluidine blue for light microscopic morphometry. Each block measured 1–4 mm² in area. The tissue samples were projected with a Pradovit projector (Fa. Leitz) directly onto a 144-point grid. All points counted in tissue not occupied by myocardium (after subtraction of points occupied by blood vessels and perivascular tissue) were expressed as a percentage of the entire tissue sample. The axis of the grid was then rotated about 45° and the determination was repeated. Only longitudinal sections at a magnification of x 250 were evaluated. One to six sections were counted per patient (average, 3.5; total, 102
sections). For each section, 288 points were counted (total 29,376 points). When results of 76 tissue sections were compared (including data of patients with coronary artery disease that are not included in the present study), a close correlation was found between the two determinations \((r = 0.97)\). When interobserver variability was tested using 51 tissue sections and two observers, the correlation coefficient was 0.93.

Statistical evaluation was done using the \(t\) test for unpaired observations. Analysis of variance\(^6\) was used when three groups were compared.

**Results**

All patients with AS had coronary arteriograms without significant obstructions. Patients with AS were divided into two groups according to EF and mean left atrial pressure. In our laboratory, the lower limit of normal for EF is 55% (2 standard deviations below the mean\(^6\)) and for mean left atrial pressure, 15 mm Hg (2 standard deviations above the mean). Patients in group 1 had an EF greater than 55% (mean 65.1 ± 8.7%, range 59–81%) and mean left atrial pressure less than 15 mm Hg (mean 10.7 ± 3.2 mm Hg, range 5–14 mm Hg). Patients in group 2 had an EF less than 55% (mean 43.8 ± 12.5%, range 21–54%) and mean left atrial pressure greater than 15 mm Hg (mean 23.3 ± 6.5 mm Hg, range 16–38 mm Hg).

**Hemodynamic Data**

The hemodynamic data for the patients with AS are given in table 1 and figure 1.

**Morphometric Data**

The morphometric data from all three groups are shown in table 2 and figure 2.

### Table 1. **Hemodynamic Data for Patients with Aortic Stenosis**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 7)</th>
<th>Group 2 (n = 12)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>70.9 ± 8.0</td>
<td>82.6 ± 12.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td>195.4 ± 10.9</td>
<td>205.5 ± 44.4</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic aortic pressure (mm Hg)</td>
<td>121.9 ± 27.5</td>
<td>115.3 ± 20.0</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic aortic pressure (mm Hg)</td>
<td>67.4 ± 16.6</td>
<td>61.8 ± 10.6</td>
<td>NS</td>
</tr>
<tr>
<td>Peak-to-peak systolic transvalvular gradient (mm Hg)</td>
<td>73.6 ± 24.5</td>
<td>89.2 ± 44.1</td>
<td>NS</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure (mm Hg)</td>
<td>21.4 ± 7.2</td>
<td>25.7 ± 6.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Right ventricular systolic pressure (mm Hg)</td>
<td>33.6 ± 8.0</td>
<td>49.2 ± 12.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Right ventricular end-diastolic pressure (mm Hg)</td>
<td>7.7 ± 2.9</td>
<td>8.4 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Aortic regurgitation (angiographic degree)</td>
<td>1.1 ± 1.1</td>
<td>3.4 ± 0.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume (ml/m(^2))</td>
<td>91.9 ± 25.7</td>
<td>145.3 ± 46.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Left ventricular muscle mass (g/m(^2))</td>
<td>148.3 ± 20.9</td>
<td>199.8 ± 43.5</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Mean circumferential fiber shortening rate (circ/sec)</td>
<td>1.2 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean normalized systolic ejection rate (vol/sec)</td>
<td>2.1 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peak systolic wall stress (dyn × 10(^4)/cm(^2))</td>
<td>399.7 ± 71.9</td>
<td>389.9 ± 56.7</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Table 2. Comparisons of Morphometric Data in Patients with Coronary Artery Disease and Patients with Aortic Stenosis

<table>
<thead>
<tr>
<th>Morphometric data</th>
<th>Coronary artery disease (controls) (n = 10)</th>
<th>Aortic stenosis Group 1 (n = 7)</th>
<th>Aortic stenosis Group 2 (n = 12)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myofibrils (%)</td>
<td>52.9 ± 4.8</td>
<td>48.4 ± 4.7</td>
<td>42.1 ± 4.9</td>
<td>NS</td>
</tr>
</tbody>
</table>
| Sarcoplasm (%)    | 21.1 ± 5.2                                  | 31.7 ± 3.1                     | 36.0 ± 5.5                      | < 0.005itional contractile function in several patients with severe muscular obstruction to right ventricular outflow tract and in chronic aortic valve disease have been reported. In some patients with atrial pressure overload due to endocardial cushion defect, loss of myofibrils was seen in right atrial myocardial cells. However, changes in intracellular volume fractions were not quantitated in these studies. In contrast, Fleischer et al. investigated transmural LV biopsy material by electron microscopic morphometry and found 52% myofibrils in myocardial cells of patients with mitral stenosis. They interpreted this value as normal for adult human patients. Our data in patients with coronary artery disease and normal wall motion resemble the data of Fleischer et al. This supports the assumption that the volume fraction of myofibrils was normal in this group. A significant reduction in the volume fraction of myofibrils was found in group 2 compared with controls, but not in group 1. Group 2 had more advanced hypertrophy and poorer LV function than group 1, which still had normal LV function. Thus, group 1 represents compensated pressure overload and group 2 represents decompensated pressure overload.

Electron microscopic morphometry has been used in the experimental animal in several studies. Sheridan et al. determined the volume fraction of myofibrils in right ventricular papillary muscles of neonatal and adult cats and found an increase from 35% to 48% with age. Page et al. reported that the normal volume fraction of myofibrils in the rat heart was 46–50%; this volume fraction increased to 53% within 1 month after supravalvular aortic constriction. In contrast, Hatt and co-workers found a significant reduction in the volume fraction of myofibrils in the rat heart 4 days after constriction of the abdominal aorta (from 59% to 50%) associated with an increased volume fraction of sarcoplasm (from 10% to 17%). Ten days after removal of aortic constriction, the volume fraction of myofibrils was not different between groups.

Figure 2. Comparison of morphometric data between controls (C), i.e., patients with moderate stenosis of the left anterior descending coronary artery and normal regional wall motion, and patients with aortic stenosis (AS) in group I (I) and group 2 (II). The volume fraction of contractile material (myofibrils) was significantly lower in group 2 than in controls, while that of sarcoplasm was higher. Fibrosis was not different between groups.
fibrils increased from 50% to 58% and that of sarcoplasm decreased from 17% to 12%. These investigators suggested that the reduction in the volume fraction of myofibrils during sudden acute pressure overload might have been the result of two opposed phenomena: an active hypertrophy (production of myofibrils) and cell damage (myofibrillar lysis); they also found that degenerative intracellular changes were more pronounced in the subendocardium than in the subepicardium of the left ventricle. In addition, the cell width was greater in the subendocardial than in the subepicardial layer (14 μ vs 11 μ). These observations revealed that hypertrophy was more advanced in the subepicardial layer of the left ventricle than in the subepicardial layer of the experimental animal studied. However, the data of Bishop and Melsen22 revealed that results of experimental studies should be interpreted with caution and cannot be extrapolated to hypertrophy of patients with chronic pressure overload. We have no data to compare subendocardial and subepicardial myocardium to clarify regional differences of hypertrophy in patients with chronic pressure overload.

LV muscle mass was higher in group 2 than in group 1. Increased muscle mass was a result of compensatory concentric hypertrophy in pressure-overloaded hearts. It is evident from investigations of hypertrophied cardiac muscle that cells do increase in size and that there is an increase of intracellular organelles.23 However, it remains unclear by what mechanism myocytes produced increased amounts of myofibrils, although sarcomere units may be added to the end of the cell.24 In the present study, we found a volume fraction of 52.9% of myofibrils in the left ventricle of control cases. If we consider 75 g/m² LV muscle mass as normal,16 and ignore interstitial fibrosis, this would result in 40 g/m² myofibrils (0.529 × 75 g/m²) in the normal left ventricle. The respective values would be 72 g/m² for group 1 and 84 g/m² for group 2 (as estimated from table 1). These two values are higher than normal (because of hypertrophy), but would be even higher if the volume fraction of myofibrils remained unchanged during development of hypertrophy, i.e., 78 and 106 g/m². Thus, a reduction in the volume fraction of myofibrils caused remarkable reduction in the amount of myofibrils in the hypertrophied hearts of patients in group 2. No evidence from the present study indicates whether reduction in the volume fraction of myofibrils was caused by decreased synthesis or by lysis of myofibrils. The latter mechanism has been suggested by several studies.1,3

No significant difference in myocardial fibrosis was found between groups 1 and 2. This observation suggests that the degree of myocardial fibrosis in pressure overload is not directly related to hypertrophy or to LV function. In patients who had coronary artery disease and normal regional wall motion, fibrosis was slightly but not significantly increased as compared with patients who had AS. The fibrotic content of LV myocardium has been determined by morphometry in studies of postmortem hearts.25, 26 These studies showed that the volume fraction of fibrosis of the LV free wall was 9% (range 5–14%) in normal hearts, but 24% (range 12–36%) in hearts with coronary artery disease. (The myocardium evaluated in hearts with coronary artery disease did not contain old transmural scars.) Our results show moderately elevated values for fibrosis as compared to the normal range in the postmortem studies, but the different preparation techniques may partly account for this difference. Therefore, we cannot definitely decide whether interstitial fibrosis was augmented in our patients or not. Blumgart et al.27 measured collagen content of human hearts and reported that only nine of 23 hypertrophied hearts had an increased level and that five of these nine had associated significant coronary artery disease. Oken and Boucek28 found that only two of 14 hypertrophied hearts had increased concentration of hydroxyproline (reflecting the amounts of collagen in myocardium), whereas in most hypertrophied hearts the hydroxyproline concentration was normal. These authors therefore concluded that in hypertrophied hearts the total amount of collagen must actually be increased to the same degree as the increase in muscle tissue. Wigle29 described five postmortem human hearts with valvular aortic stenosis and increased fibrosis, especially in the subendocardial layer of the left ventricle in three of them. One heart, however, had calcareous emboli in the coronary arteries. In the present study we did not subdivide the tissues into subendocardial and subepicardial layers and we cannot rule out the possibility that subendocardial fibrosis may have been higher in group 2 than in group 1. However, in another study we found slightly higher degree of fibrosis in subendocardial than in subepicardial tissue samples of 13 pressure-overloaded hearts.8

In conclusion, the volume fraction of myofibrils was reduced in the LV tissue biopsy specimens of patients with decompensated pressure overload and advanced hypertrophy and was probably normal in patients with compensated pressure overload. Interstitial fibrosis was not different between AS with normal and impaired LV function. We therefore consider a reduction in the volume fraction of myofibrils to be the myocardial factor in chronic pressure overload.

References

5. Schwarz F, Flameng W, Thiedemann KU, Schaper W,

11. Schlepper M: Effect of coronary stenosis on myocardial function, ultrastructure and aortocoronary bypass graft hemo-
dynamics. Am J Cardiol 42: 193, 1978


9. Kennedy JW, Trenholme SE, Kasser IS: Left ventricular volume and mass from single-plane cineangiogram. A com-
parison of anteroposterior and right anterior oblique methods. Am Heart J 80: 343, 1970


12. Falsetti HL, Mates RE, Grant C, Greene DG, Bunnell IL: Left ventricular wall stress calculated from one-plane cinean-


18. Fleischer M, Warmuth H, Backwinkel KP, Themann H: Ultrastructural morphometric analysis of normally loaded myocar-

diovasc Res 11: 536, 1977

chemical and stereological measurements in normal and hyper-


27. Blumgart H, Gilligan D, Schlesinger M: The degree of myocardial fibrosis in normal and pathologic hearts as estimated chemically by the collagen content. Trans Assoc Am Physicians 55: 313, 1940


Reduced volume fraction of myofibrils in myocardium of patients with decompensated pressure overload.
F Schwarz, J Schaper, D Kittstein, W Flameng, P Walter and W Schaper

Circulation. 1981;63:1299-1304
doi: 10.1161/01.CIR.63.6.1299
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/63/6/1299.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org//subscriptions/