Hypertrophic cardiomyopathy (HCM) is a genetically transmitted disease of the sarcomeres, characterized phenotypically by an inappropriate thickness of the interventricular septum and less frequently of the free left ventricular wall of a nondilated ventricle (1,2). Apart from the striking pathologic and molecular findings of the myocardium, recent reports indicate that the coronary microcirculation is disturbed in HCM. Atypical and typical chest pain are common symptoms in patients with HCM. This is also the case in the context of angiographically normal epicardial coronary arteries (3,4). Thallium-201 imaging revealed reversible perfusion defects both within the ventricular wall and especially in the subendocardium (5). These were found to be associated with increased myocardial lactate production, metabolic evidence of ischemia (6). Previous reports indicate the important role of myocardial ischemia in the development of symptoms and in relation to prognosis (7,8). Several potential mechanisms for myocardial ischemia have been proposed, including septal perforator artery compression (9), abnormalities in diastolic relaxation and filling that may impede myocardial oxygen delivery by increased extravascular compressive forces (1,4), increased oxygen demand due to dynamic outflow tract gradients (10), myocardial bridges, abnormal intramyocardial small coronary arteries (11) and an inadequate capillary density in relation to the increased myocardial mass (12).

Furthermore, there is growing evidence that abnormalities of intramural coronary arteries may be the cause of myocardial ischemia (13), especially as arterioles mainly regulate intramyocardial blood flow (14,15) and their cross-sectional area represents the intramural coronary resistance (16).

The present investigation was designed to study the structural alterations of preterminal intramural arterioles in the

Objectives. The study was designed to investigate the architecture of subendocardial arterioles of patients with hypertrophic cardiomyopathy (HCM) and angina pectoris with respect to coronary vasodilator reserve.

Background. There is growing evidence that the coronary microvasculature is abnormal in HCM. Arterioles, which mainly regulate intramyocardial blood flow, are especially suspect.

Methods. Thirteen patients with HCM (50.1 ± 12.6 years old, mean value ± SD) were studied after exclusion of any relevant coronary stenoses. Subendocardial arterioles (density [n/mm²], wall area [μm²], percent lumen area [%lumen], periarteriolar collagen area [μm²], myocyte diameter (μm) and interstitial collagen fraction (Vv%) were evaluated by means of stereologic morphometry of transvenous biopsy samples. Coronary blood flow was measured quantitatively with the inert chromatographic argon method at basal conditions and after dipyridamole (0.5 mg/kg body weight over 4 min intravenously), and coronary vasodilator reserve was calculated as the ratio of coronary resistance at basal conditions and after pharmacologic vasodilation. Data from five normotensive subjects (45.4 ± 11 years old, p = NS) served as control data.

Results. Arteriolar density was diminished by 38% (p = 0.004) and %lumen by 13% (p = 0.009) in patients with HCM compared with control subjects. Coronary reserve was impaired in patients with HCM (2.28 ± 0.6 vs. 5.34 ± 1.49, p = 0.003) because of higher coronary resistance after vasodilation (0.48 ± 0.14 vs. 0.22 ± 0.06 mm Hg × min × 100 g/ml, p = 0.004). Coronary vasodilator reserve correlated with arteriolar density (r = +0.47, p = 0.045) and with %lumen (r = 0.65, p = 0.003).

Conclusions. In HCM, the architecture of preterminal subendocardial arterioles is altered by a reduced total cross-sectional lumen area, corresponding to an impaired coronary vasodilator capacity that may predispose to myocardial ischemia.

(J Am Coll Cardiol 1998;31:1089–96) ©1998 by the American College of Cardiology
right septal subendocardium by morphometric methods in patients with hypertrophic cardiomyopathy and angina pectoris.

Methods

Patient selection. We studied 13 consecutive patients with a clinical and echocardiographic diagnosis of HCM (septum >15 mm, septum/posterior wall ratio >1.3 in the absence of other known causes of left ventricular hypertrophy) (1) (Table 1). All patients reported typical or atypical chest pain and exertional dyspnea (New York Heart Association functional class II to IV, mean value 2.47 ± 0.85), four patients complained of presyncope. All patients had been previously treated with beta-blocking agents (n = 4) or a calcium channel blocking drug (verapamil, n = 9) without significant amelioration of symptoms. All patients underwent cardiac catheterization to assess the presence and severity of left ventricular outflow tract obstruction and suitability for operation (septal myectomy). Angiography excluded coronary artery stenoses of >20% in each of the patients.

Table 1. Clinical, Hemodynamic and Echocardiographic Data of Patients With Hypertrophic Cardiomyopathy and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>HCM Group</th>
<th>Control Group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/3</td>
<td>3/2</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>50.1 ± 12.6</td>
<td>45.4 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>Hemodynamic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>127.2 ± 13.5</td>
<td>124.0 ± 17.1</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>68.6 ± 5.9</td>
<td>73 ± 10.3</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>71.4 ± 9.9</td>
<td>74.4 ± 10.7</td>
<td>NS</td>
</tr>
<tr>
<td>LV obstruction (gradient &gt;30 mm Hg at basal conditions)</td>
<td>4/13</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>66 ± 5</td>
<td>62 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac index (liter/min per m²)</td>
<td>2.98 ± 0.76</td>
<td>3.1 ± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>19.2 ± 9.6</td>
<td>10.0 ± 4.5</td>
<td>0.053</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septum (mm)</td>
<td>24.7 ± 6.0</td>
<td>10.8 ± 1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Septum/posterolateral wall ratio</td>
<td>2.24 ± 0.68</td>
<td>0.98 ± 0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>41.8 ± 4.9</td>
<td>48.0 ± 4.1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data presented are mean value ± SD or number of subjects. HCM = hypertrophic cardiomyopathy; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; LVEDP = left ventricular end-diastolic pressure; LVEF = left ventricular ejection fraction. Five normotensive patients (mean age 45 ± 11 years, p = NS) without echocardiographic evidence of HCM or dilative cardiomyopathy and without valvular heart disease, and normal epicardial coronary arteries were investigated to exclude unexplained chest pain (Table 1).

Echocardiographic studies. A transthoracic M-mode echocardiogram, guided by a two-dimensional technique, was obtained using a Toshiba SSH 140. M-mode echocardiography was performed with a 2.25- or 3.5-MHz transducer. Basal septal and posterior wall thicknesses were determined from the M-mode echocardiogram according to the American Society of Echocardiography (17), and the septum/posterior wall ratio was calculated.

Measurement of central hemodynamic variables. Before measuring the coronary circulation, the pressures in the pulmonary artery, pulmonary capillary wedge position, right atrium and aorta were measured with fluid-filled catheters. Cardiac output was determined by thermodilution as the mean value of at least three measurements. Quantitative biplane ventriculography was performed (30° right anterior oblique, 60° left anterior oblique) by injecting 40 ml of nonionic contrast medium (Ultravist-370, Schering Inc., Berlin, Germany) into the left ventricle through a pigtail catheter at a speed of 12 ml/s. Left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were determined semiautomatically by the AVD system (Siemens Inc., Munich, Germany). The left ventricular ejection fraction was calculated as (EDV – ESV)/EDV × 100, and was >60% in each of the patients and the controls. These investigations as well as the coronary angiography were carried out between 3 and 5 days before the measurement of the coronary circulation.

Evaluation of coronary vasodilator capacity. All medication was discontinued at the time of admission to the clinic. Coronary blood flow was quantitatively measured using the inert chromatographic argon method under baseline conditions and after pharmacologic vasodilation, which occurs as follows: gas chromatographic determination of the argon concentration of blood samples taken simultaneously from the coronary sinus and the descending aorta while the patient is breathing an oxygen-argon mixture (21% oxygen, 79% argon). The aortic pressure was measured with a fluid-filled multipurpose catheter in the descending aorta. Coronary vascular resistance was calculated as the coronary perfusion pressure (coronary perfusion pressure equals mean systemic arterial pressure minus mean right atrial pressure) divided by coronary blood flow. Coronary reserve was calculated as the ratio of coronary vascular resistance under basal conditions to coronary resistance after coronary vasodilation by dipyridamole. To avoid metabolic effects leading to an increase in left ventricular obstruction, dipyridamole was given in a low dose (0.5 mg/kg body weight) over a period of 4 min. Myocardial oxygen consumption per unit weight of myocardium was determined as the product of coronary blood flow per unit weight of myocardium and the arterial-venous (coronary sinus) oxygen difference. By this method, information about those regions that drain into the coronary sinus can be derived. Advantages
and disadvantages of the method have been discussed previously (18).

**Morphologic investigation.** Transvenous endomyocardial biopsy was performed under biplane fluoroscopic control to gain at least 6 samples from the right basal septum of each patient. In the normal heart at least one arteriole was found in a subendocardial biopsy sample of >1.22 mm² (19). For an appropriate estimate we evaluated five biopsy samples, each having >1.22 mm² of tissue section area, for each patient (19).

Samples were immediately fixed in 4% buffered formalin, embedded in paraffin and numbered. Assuming the myocardial structure to be partially anisotropic, special care has to be taken to generate isotropic conditions for the sectioning of biopsy samples that allow the application of common rules of stereology (19,20). Therefore, each biopsy sample was embedded into a sphere and rolled in random directions; thus generating an isotropic uniform random section, which allows measurement of the myocardial structure without bias (19,20). Samples were immediately fixed in 4% buffered formalin, embedded in paraffin and numbered. Assuming the myocardial structure to be partially anisotropic, special care has to be taken to generate isotropic conditions for the sectioning of biopsy samples that allow the application of common rules of stereology (19,20). Therefore, each biopsy sample was embedded into a sphere and rolled in random directions; thus generating an isotropic uniform random section, which allows measurement of the myocardial structure without bias (19,20). Serial sections (n = 5) of each biopsy sample were cut with a thickness of 4 to 5 μm, and stained in hematoxylin-eosin (HE), elastica van Gieson (EvG) and picro-Sirius red. Each section area was planimetrically measured by a semiautomated image analysis system (Quantimet 570, Cambridge, Leica). Differentiation between arterioles and veins was done according to Weiss and Conway (21,22) in the EvG-stained section, where arterioles are characterized by a continuously stained yellow, tunica media, a black lamina elastica, and a yellow-red tunica adventitia. All profiles of intramyocardial arterioles were counted independently from their form or direction. The mean number of arterioles per square millimeter in the right septal subendocardium for patients with HCM and for control subjects was calculated from the mean number of each patient and each control subject, determined from the arteriolar density per square millimeter in sections >1.22 mm². Morphometric analysis of the single arteriolar wall was performed on strictly transversely cut arteriolar profiles, resulting in a circular appearance of the vessel, as previously reported (22) (Fig. 1).

Collagen was determined using picro-Sirius red, which specifically stains collagen reddish and has been found to correlate with the biochemical measurements of collagen content (23). The following variables were determined for each patient: 1) **Mean myocyte diameter across the nucleus** (μm): the mean of 20 measurements in each biopsy in HE stain at 400× magnification giving a mean of 100 measurements/patient. 2) **Mean volume density (Vv%) of interstitial collagen**: the mean of repeated measurements with 10 randomly distributed measurements in one biopsy, at 100× magnification using the color image analysis system (Quantimet 570, Cambridge), giving a mean total of 50 measurements/patient. 3) **Numerical density of arterioles (n/mm²)** in EvG stain at 400× magnification. 4) **Quantification of the wall of circular arteriolar profiles** in EvG at a 800× magnification from all circular arteriolar profiles: a) **Wall area (μm²) and percent lumen area (%lumen)**: the lamina elastica interna, including the intima, and the outer border of the tunica media, marked by the tunica adventitia, were surrounded by the computerized system, giving the absolute area of the wall and the lumen area. The %lumen was derived from lumen area/(lumen area + wall area) × 100 (according to ref. 13). b) **Outer arteriolar diameter**: from the total vascular area the outer diameter of each arteriole was calculated, excluding the adventitia (according to ref. 22). c) **Periarteriolar collagen (adventitia) area**: as the periarteriolar collagen area cannot be distinguished from the adventitial collagen, total reddish stained collagen, surrounding each circular arteriole, was measured by the computerized color detection system, avoiding larger spaces.

The study protocol was approved by the ethics committee of Heinrich-Heine University of Düsseldorf. Informed consent was obtained from all patients before each investigation.
Table 2. Coronary Hemodynamic Variables at Basal Conditions and After Vasodilation in Patients With Hypertrophic Cardiomyopathy and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>HCM Group (n = 13)</th>
<th>Control Group (n = 5)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>71.4 ± 9.9</td>
<td>74.7 ± 10.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>86.7 ± 12.3</td>
<td>90.6 ± 8.0</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min per 100 g)</td>
<td>91.6 ± 20.0</td>
<td>81.2 ± 16.8</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary resistance (mm Hg × mm x 100 g/ml)</td>
<td>1.03 ± 0.24</td>
<td>1.11 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Myocardial oxygen consumption (ml/min per 100 g)</td>
<td>10.8 ± 1.98</td>
<td>10.4 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>After vasodilation (dipyridamole 0.5 mg/kg body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>86.5 ± 14.9</td>
<td>89.6 ± 6.1</td>
<td>NS</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>83.5 ± 13.5</td>
<td>85.6 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min per 100 g)</td>
<td>192.0 ± 60.7</td>
<td>392.4 ± 140.9</td>
<td>0.007</td>
</tr>
<tr>
<td>Coronary resistance (mm Hg × mm x 100 g/ml)</td>
<td>0.477 ± 0.143</td>
<td>0.218 ± 0.063</td>
<td>0.004</td>
</tr>
<tr>
<td>Myocardial oxygen consumption (ml/min per 100 g)</td>
<td>14.2 ± 3.8</td>
<td>16.1 ± 5.6</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary reserve</td>
<td>2.28 ± 0.60</td>
<td>5.34 ± 1.49</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Coronary vasodilator reserve is impaired because of inadequate reduction of coronary resistance after pharmacologic intervention. Data presented are mean value ± SD. HCM = hypertrophic cardiomyopathy.

Statistical analysis. Descriptive data are expressed as mean value ± SD. For interobserver and intraobserver variability the coefficient of variance and the coefficient of error (calculated as coefficient of variance/√n) were given. The Wilcoxon test, Mann-Whitney U test and linear regression analysis, according to Pearson, were used where appropriate. Statistical significance was inferred at a two-tailed p value of ≤0.05. The analysis was done on the SPSS-PC package (Version 4.0).

Results

Clinical data. Major clinical data are given in Table 1. The mean age of patients and control subjects did not differ. In four patients, left ventricular outflow tract obstruction was >30 mm Hg. Echocardiographically all patients had an irregular hypertrophied septum and the left ventricular end-diastolic diameter was small. Cardiac index, heart rate and systolic and diastolic blood pressure were not significantly different in both groups. Left ventricular end-diastolic pressure was higher in the HCM group, but did not reach significance (p = 0.053).

Coronary hemodynamic variables. There was no significant difference between coronary blood flow and coronary resistance at basal conditions between patients with HCM and control subjects (Table 2). Furthermore, myocardial oxygen consumption showed no difference at rest for both groups. In the four patients with left ventricular obstruction (>30 mm Hg) at basal conditions, coronary blood flow (99.5 ± 17.7 ml/min per 100 g), coronary resistance (0.898 ± 0.136 mm Hg × mm × 100 g/ml) and myocardial oxygen consumption (10.56 ± 0.64 ml/min/100 g) did not differ significantly from control values. After dipyridamole administration, in control subjects coronary blood flow was significantly higher and coronary resistance was significantly lower than in patients with HCM. Thus, coronary reserve was significantly impaired in patients with HCM.

Morphologic findings. In the right septal subendocardium in HCM, myocytes showed a significant hypertrophy, collagen content was increased and arteriolar density was significantly reduced (Table 3). The mean diameter of arterioles was not

Table 3. Morphometric Findings in the Right Septal Subendocardium

<table>
<thead>
<tr>
<th></th>
<th>HCM Group (n = 13)</th>
<th>Control Group (n = 5)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteriolar density (n/mm²)</td>
<td>1.02 ± 0.37</td>
<td>1.65 ± 0.23</td>
<td>0.004</td>
</tr>
<tr>
<td>Arteriolar diameter (μm)</td>
<td>18.4 ± 4.2</td>
<td>18.1 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Arteriolar wall area (μm²)</td>
<td>212.6 ± 119.0</td>
<td>180.2 ± 47.2</td>
<td>NS</td>
</tr>
<tr>
<td>%lumen area</td>
<td>23.89 ± 2.003</td>
<td>27.51 ± 2.23</td>
<td>0.009</td>
</tr>
<tr>
<td>Periarteriolar collagen area (μm²)</td>
<td>125.7 ± 82.3</td>
<td>124.6 ± 69.5</td>
<td>NS</td>
</tr>
<tr>
<td>Myocyte diameter (μm)</td>
<td>16.3 ± 1.54</td>
<td>13.0 ± 0.88</td>
<td>0.002</td>
</tr>
<tr>
<td>Collagen volume density (Vv%)</td>
<td>4.2 ± 1.56</td>
<td>1.93 ± 0.62</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Arterioles have a lower density and a reduced percent lumen area (%lumen). Myocyte hypertrophy and interstitial fibrosis, in terms of increased collagen volume density (Vv%), are present. Data presented are mean value ± SD. HCM = hypertrophic cardiomyopathy.

Figure 2. With increasing hypertrophy of myocytes (determined by measuring their diameter), arteriolar density is reduced. Circles = control subjects (n = 5); squares = patients with HCM (n = 13).
significantly different in HCM patients and controls, nevertheless their range for the group of patients with HCM (13.8 to 26.7 μm) was larger than in control subjects (15.12 to 19.53 μm). Quantitative data of %lumen revealed a significant reduction in HCM (Table 3 and Fig. 3); the mean arteriolar wall area and mean periarteriolar collagen area did not differ significantly between patients with HCM and control subjects.

Univariate analysis revealed a significant negative correlation between density of arterioles and myocyte size (Fig. 2). Furthermore, the density of arterioles as well as %lumen correlated significantly with minimal coronary resistance and coronary reserve (Fig. 4 and 5).

Reproducibility of morphometric data. The biopsy area in patients with HCM was comparable with the data of control subjects (1.53 ± 0.43 mm² vs. 1.68 ± 0.51 mm²), and the total number of evaluable circular arteriolar profiles for determination of wall parameters from different sections were not significantly different (13.6 ± 8.8 vs. 12.6 ± 5.1 per patient).

Intra- and interindividual observer variability was determined according to a previously reported protocol (19,24) by repeating all measurements of 10 randomly chosen biopsy samples by one observer (M.M.) (intraobserver variability) and comparing the values of the first measurement with those of a second observer (B.S.). There were no significant differences for the mean of any parameter between the first and second measurement of the first observer (intraobserver) and between those of the second observer (interobserver). Results are given in Table 4, with the mean value and SD of the absolute differences of the observations as well as the coefficient of variance expressed as the ratio of SD of the absolute differences to the mean of the observation in percent (20,24).

Discussion

Several reports from different groups have led to the belief that coronary vasculature is abnormal in patients with HCM. Our main finding is a reduced density and a diminished %lumen of preterminal arterioles in the subendocardium of patients with HCM that correlates with a diminished coronary vasodilator capacity.

Vasodilator capacity in HCM. In fact, there are many reports indicating perfusion abnormalities after exercise (6,25,26), during pacing-induced tachycardia (4,12) and after pharmacologic vasodilation (27) in patients with as well as without history of chest pain who have HCM. In our study of patients with HCM and chest pain, coronary vasodilation after dipyridamole was significantly reduced as compared with control subjects. Oxygen consumption per 100 g of myocardium was not significantly different for patients with obstructive and nonobstructive HCM compared with control subjects, indicating that an increased metabolic demand did not explain the limitation of coronary reserve in our patients. Cannon et al. (10) reported a significantly increased myocardial oxygen consumption and a higher coronary blood flow at rest in patients with marked left ventricular outflow tract obstruction compared with patients with nonobstructive cardiomyopathy. Nevertheless, in this study at a maximal paced heart rate of 150 beats/min, most patients had angina pectoris and severe metabolic evidence of ischemia, whereas coronary resistance was not significantly different between both groups. This supports our finding that inadequate reduction of coronary resistance is an important factor for compromised coronary vasodilator reserve in HCM. Furthermore, our data are in accordance with those of Camici et al. (27) who reported an impaired decrease
in coronary resistance after dipyridamole in patients with HCM by means of nitrogen-13 ammonia and dynamic positron emission tomography compared with control subjects. Coronary reserve was reduced in patients with as well as in those without angina pectoris, but there was a tendency to a more reduced coronary reserve in those with angina, indicating a more serious disturbance in coronary microcirculation.

A reduced coronary vasodilator capacity after dipyridamole has been partially explained by the increased extravascular compressive forces in HCM (4). In our study, left ventricular end-diastolic pressure tended to be higher, but did not reach significance between patients with HCM and control subjects, indicating the importance of other factors for increased minimal coronary resistance. From experimental data it is well known that dipyridamole, through adenosine, dilates intramu-
Altered architecture of intramural arterioles in HCM. Maron et al. (11) qualitatively reported from autopsy studies abnormal intramural coronary arteries in 40 of 48 patients with HCM. Abnormal arteries were characterized by a thickening of the vessel wall and a decrease in luminal size, which were more frequently found in the interventricular septum than in the anterior and posterior left ventricular free wall. Nevertheless, in most patients only a small part of the arterial profiles were abnormal, indicating a segmental character in the expression of the abnormal intramural coronary arterioles.

Furthermore, the mean arterial diameter was 300 μm, with a range of 50 to 1,300 μm, whereas qualitative abnormalities of smaller arterioles were not reported.

In this quantitative morphometric investigation we did not find a statistically significant thickening of the preterminal arteriolar wall in HCM, but %lumen was significantly reduced, indicating eutrophic vascular remodeling (29). Our data are in accordance with those of Tanaka et al. (13), reporting a reduced %lumen of arterioles and arteries, indicating the functional and prognostic importance of vascular remodelling of intramural coronary vessels in HCM (13). The significant correlation between %lumen and coronary reserve in our study supports this concept.

In this study, arteriolar density in the subendocardium was found to be reduced, correlating inversely with coronary resistance and concordantly with coronary reserve after pharamacologic vasodilation. This finding gives further evidence of a reduced total cross-sectional arteriolar lumen area that is probably an important factor for the reported clinical and metabolic signs of myocardial ischemia, especially in the subendocardium (5–7). Furthermore, the observed increased content of collagen in the subendocardium could be the structural consequence of ischemia, as previously discussed (11,13).

It should be noted that an inadequate relation between arterioles and hypertrophied myocytes is apparent in HCM. Myocyte hypertrophy correlated negatively with the density of arterioles, which may point to an enhanced spreading of the arteriolar bed. Perfusion abnormalities have been reported by scintigraphic studies (5–7) as well as by positron emission tomography studies (26) to a larger extent in the hypertrophied than in the nonhypertrophied myocardium. Data from pressure-induced hypertrophy reported an inadequate growth of arterioles in the early process of experimental hypertrophy (30), which was the most plausible cause for a reduced coronary reserve. In human adult hearts, Rakusan et al. (31) found an inadequate density of capillaries in left ventricular pressure-overload hypertrophy. Besides an inadequate growth of the intramural coronary arteries, a true loss of arteriolar segments could be a further explanation, as reported by Anversa et al. (32) in an experimental model.

Further investigations at the cellular and molecular level seem necessary to clarify the processes leading to alterations in the architecture of the intramyocardial coronary tree, regarding the single arteriolar wall/lumen ratio and the number and length of its branches.

Study limitations. Our data are restricted to the subendocardium of the right ventricular septum. Thus, information about the rest of the myocardium cannot be given. Nevertheless, the investigated part is involved in the process of hypertrophy as evidenced by hypertrophied myocytes. Secondary processes (e.g., by increased pressure or contact with the mitral leaflet) can be excluded. Sampling of arterioles in our study depended on the depth gained by the bioptome, which was comparable in patients and control subjects and thus excludes bias achieved by ranking of arterioles because all arterioles were investigated. Nevertheless, the observed greater variability regarding arteriolar wall area and diameter between patients with HCM and control subjects might indicate a segmental process for arteriolar wall hypertrophy or the presence of an afflicted minority of patients with true arteriolar wall thickening. Additionally, even if we could demonstrate reduced %lumen and diminished density of arterioles in HCM, correlating both with increased minimal coronary resistance, total maximal dilated arteriolar lumen area for the whole heart cannot be sufficiently concluded from our data. The influence of “stenotic intramural coronary arteries” residing above preterminal arterioles and potentially contributing to a diminished vasodilator capacity could not be analyzed because arteries were not obtained. Considering the relatively low correlations between vascular alterations in the subendocardium and reduced coronary reserve one might expect additional contributing factors.

### Table 4. Intraobserver and Interobserver Variabilities of Morphometric Investigations

<table>
<thead>
<tr>
<th>Observer Variabilities</th>
<th>Intraobserver</th>
<th>Interobserver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte diameter (μm)</td>
<td>0.24 ± 0.09</td>
<td>0.27 ± 0.12</td>
</tr>
<tr>
<td>Arteriolar area (μm²)</td>
<td>0.20 ± 0.56</td>
<td>0.22 ± 0.78</td>
</tr>
<tr>
<td>Percent lumen area (%)</td>
<td>3.0 ± 1.4</td>
<td>3.0 ± 2.8</td>
</tr>
<tr>
<td>Periarteriolar collagen</td>
<td>9.8 ± 20.3</td>
<td>14 ± 60</td>
</tr>
<tr>
<td>Arteriolar density (n/mm²)</td>
<td>0.1 ± 0.095</td>
<td>0.2 ± 0.27</td>
</tr>
<tr>
<td>Volume density of collagen (Vv%)</td>
<td>0.2 ± 0.63</td>
<td>0.38 ± 1.64</td>
</tr>
</tbody>
</table>

Values are expressed as mean value ± SD of the absolute difference of the observations (CV = coefficient of variance; SD of the absolute difference/mean of observation) and as coefficient of error (CE).
including the endothelium, in regard to coronary vasodilator reserve. On the other hand, vascular remodeling and reduced arteriolar density might be additive factors, which might correlate better to functional data when taken together.

We thank Stefanie Wolff for skilful assistance and preparation of the morphologic material, and Prof. Dr. Emeritus Waldemar Hort for revising and discussing the morphologic techniques and the manuscript.

References