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## Decreased Coronary Flow Reserve in Hypertrophic Cardiomyopathy Is Related to Remodeling of the Coronary Microcirculation

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**Background**—Ischemia occurs frequently in hypertrophic cardiomyopathy (HCM) without evidence of epicardial stenosis. This study evaluates the hypothesis that the occurrence of ischemia in HCM is related to remodeling of the coronary microcirculation.

**Methods and Results**—End-diastolic septal wall thickness was significantly increased in patients with HCM ( $25.8 \pm 2.9$  mm) in comparison with cardiac transplant recipients (control subjects:  $11.4 \pm 3.0$  mm;  $P < 0.05$ ). Although the diameter of the left anterior descending coronary artery was similar in both groups ( $3.0 \pm 0.8$  versus  $3.0 \pm 0.5$  mm,  $P = \text{NS}$ ), the coronary resistance reserve ( $\text{CRR} = \text{CRR}_{\text{basal}} / \text{CRR}_{\text{hyperemic}}$ ), corrected for extravascular compression (end-diastolic left ventricular pressure), was reduced to  $1.5 \pm 0.6$  in HCM ( $P < .05$ ; control,  $2.6 \pm 0.8$ ). Arteriolar lumen (AL) divided by wall area was lower in HCM ( $21 \pm 5\%$  versus  $30 \pm 4\%$ ;  $P < .05$ ), and capillary density tended to decrease (from  $1824 \pm 424$  to  $1445 \pm 513$  per  $\text{mm}^2$ ,  $P = .11$ ) in HCM. CRR was linearly related to normalized AL according to the formula  $\text{CRR} = 0.1 \text{ AL} - 0.45$  ( $r = .57$ ;  $P < .05$ ). Further analysis revealed that CRR, AL, and capillary density were all linearly related to the degree of hypertrophy.

**Conclusions**—Decrements in CRR were related to changes of the coronary microcirculation. Both the decrease in CRR and these changes in the coronary microcirculation were related to the degree of hypertrophy. All these factors might contribute to the well-known occurrence of ischemia in this patient group. (*Circulation*. 1998;97:230-233.)

**Key Words:** cardiomyopathy ■ arteries ■ capillaries ■ ischemia

Anginal symptoms and signs of ischemia occur frequently in patients with HCM without detectable lesions of the major epicardial arteries,<sup>1-5</sup> suggesting that the presence of ischemia is the result of abnormalities of the coronary microcirculation. Indeed, postmortem analysis of HCM hearts showed the existence of arterioles with abnormally thick walls.<sup>6,7</sup> Furthermore, in experimentally induced hypertrophy, it has been shown that the hypertrophic process is accompanied not only by decrements in CFR but also by structural changes in the coronary microcirculation (coronary remodeling), including a decreased capillary density.<sup>8-10</sup> The resulting increased diffusion distances for oxygen and the disturbed perfusion of the capillary bed have been forwarded as an explanation for ischemia.<sup>8-10</sup>

Although the CFR is decreased in HCM, in accordance with the findings of experimentally induced hypertrophy,<sup>9,10</sup> it is unknown at present whether the decreased CFR is related to the abnormal arterioles and whether a decrease in capillary density accompanies this decrease in AL. To that end, we measured CFR in combination with a quantitative analysis of AL, wall area and capillary density in myocardial tissue obtained during surgery (HCM) and obtained from endomyocardial biopsies (HTx).

### Methods

#### Subjects and Protocol

Studies were performed in a group of patients with hypertrophic obstructive cardiomyopathy (HCM;  $n = 10$ ) who were referred for cardiac catheterization. The control group consisted of asymptomatic cardiac transplant recipients (HTx group;  $n = 8$ ) undergoing follow-up coronary angiography after transplantation. Informed consent was obtained from all patients. Patients in the HCM group were symptomatic (NYHA class II or III) despite  $\beta$ -blockade therapy ( $n = 5$ ) or therapy with calcium antagonists ( $n = 5$ ). These patients were considered candidates for surgery (myotomy/myectomy). Medical therapy was continued in both groups. Right heart catheterization was performed with a 7F balloon-tipped flow-directed thermodilution catheter. A 7F temporary pacemaker was positioned into the right atrium. Left heart catheterization was carried out, after which left ventricular angiography and coronary arteriography were performed with standard techniques. A 0.014-in Doppler guidewire with a floppy distal end (Cardiometrics, Inc) was introduced through an 8F guiding catheter and positioned at the midsegment of the LAD to measure Doppler flow velocity at rest and after hyperemia. In both groups, hearts were paced at a constant heart rate of 100 bpm to avoid metabolic vasodilatation during determination of the CFR. After optimization of the settings of the velocity signal and 3 to 5 minutes after intracoronary injection of a bolus of 2 to 3 mg isosorbide dinitrate, baseline recordings of flow velocity and perfusion pressure were collected and digitized at a sample rate of 125 Hz for off-line

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### Selected Abbreviations and Acronyms

AL	= normalized arteriolar lumen
CFR	= coronary flow reserve
CRR	= coronary resistance reserve
HCM	= hypertrophic cardiomyopathy
HTx	= cardiac transplant
LAD	= left anterior descending coronary artery

analysis. Maximal hyperemia was induced by an intracoronary bolus injection of 18  $\mu\text{g}$  adenosine.<sup>10</sup>

### Doppler Measurements

The sample volume of the Doppler wire was positioned at a distance of 5.2 mm from the transducer and was  $\approx 2.25$  mm wide. After power spectral analysis based on a fast Fourier transform algorithm, the maximal Doppler shift (kHz) was automatically tracked and converted to the instantaneous velocity values (cm/s). CFR was defined as hyperemic divided by basal velocity ( $V_{\text{cor}}$ ). Coronary resistance was defined as  $(P_{\text{ao}} - P_{\text{ed}}) / V_{\text{cor}}$ , where  $P_{\text{ao}}$  is aortic pressure and  $P_{\text{ed}}$  is end diastolic pressure.  $P_{\text{ed}}$  was subtracted to account for increments in extravascular compression. CRR was defined as the ratio of basal divided by hyperemic resistance.

### Quantitative Angiographic Measurements

A validated on-line analysis system operating on digital images (ACA-DCI, Philips<sup>11</sup>) was used during the catheterization procedure. With this system, the end-diastolic diameter of the LAD was determined in the segment of the LAD in which the sample volume of the Doppler wire was located.

### Echocardiographic Measurements

Two-dimensional echocardiographic studies were performed (HP Sonos 1500) with the heart being visualized from standard cross-sectional planes while images were recorded on videotape (VHS) for off-line analysis. Septal wall thickness was measured in diastole from both the parasternal short-axis and long-axis views. From the recordings on videotape, representative stop-frames from the various cross-sectional planes were acquired to determine septal wall thickness with the aid of a computer and a dedicated software program. To obtain an average for septal wall thickness, the various cross-sectional planes were pooled. One patient from the control group was not analyzed because of insufficient image quality. Thickness of the septal wall for the HCM and the control groups was defined as the degree of hypertrophy.

### Histological Measurements

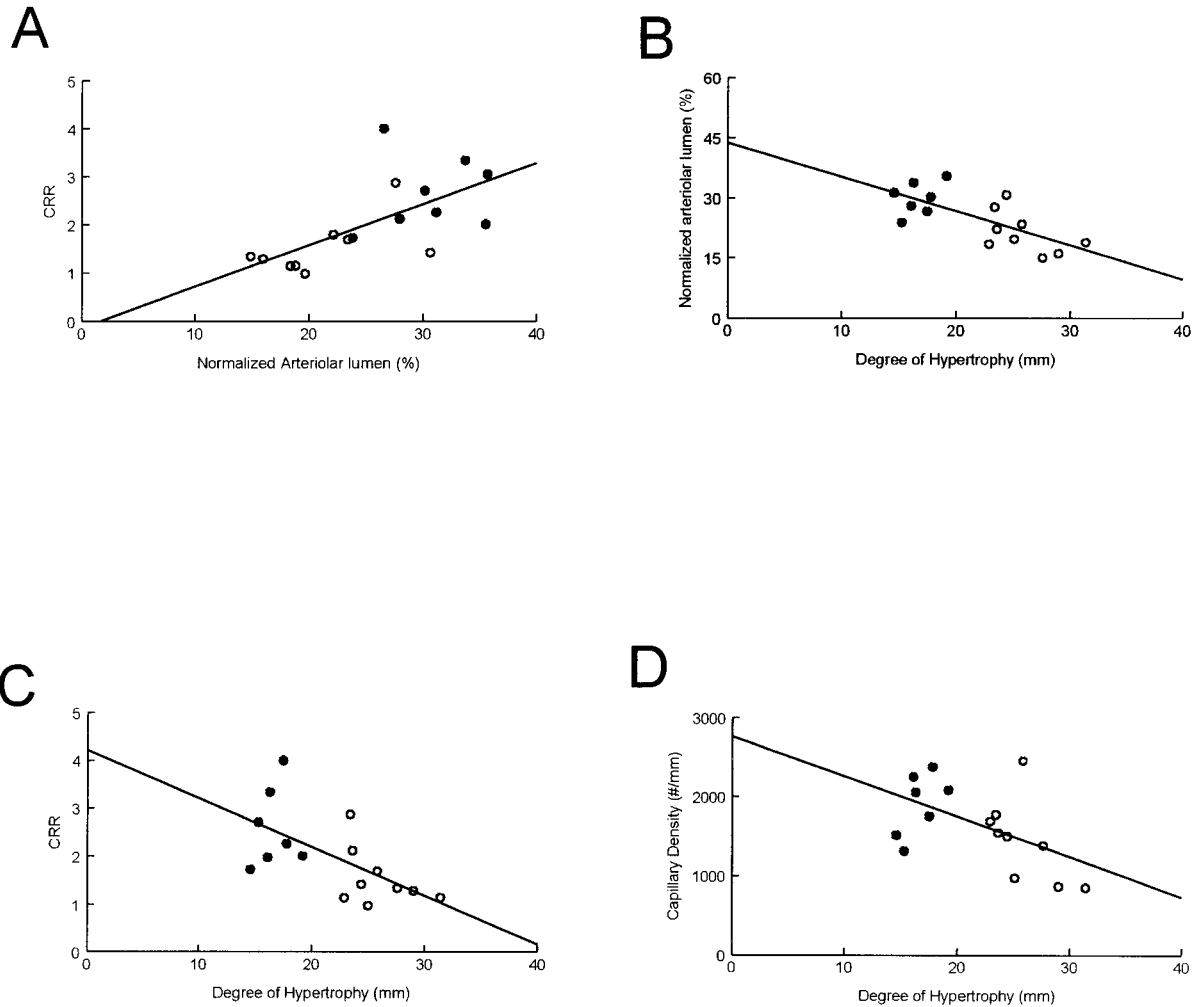
The myocardial tissues from the HCM group ( $n=9$ ) and from the control group ( $n=8$ ) were obtained from surgical myectomy (left ventricular septal tissue; weight, 0.3 to 1 g) and myocardial biopsies (left ventricular septal tissue; weight, 0.5 to 1 mg), respectively. During catheterization, one HCM patient presented without a subvalvular pressure gradient and was not operated on. The tissue was fixed with paraaldehyde and immersed in 10% buffered formalin. van Gieson staining was used for identification and analysis of intramyocardial small arteries. Arterioles were identified on the basis of the appearance of a layer of media and diameter  $<100 \mu\text{m}$ . Only arterioles with round cross sections and without side branches were analyzed. Capillaries were identified with specific antibodies (CD34) against endothelium. Quantitative morphometric analysis of the histological sections occurred with an in-house-developed software program applied to a morphometric system (Clemex Technology Inc) that calculated density of capillaries (capillaries per square millimeter), taking tissue shrinkage into account. Five cross sections per patient ( $\approx 1000$  capillaries) were analyzed. In addition, software was available that allowed us to trace the arteriolar lumen-intima and adventitia-media borders, which defined the lumen and wall thickness regions. The areas of these regions were obtained from the number of pixels in

the two regions. Normalized wall area is given by circular wall area/(lumen area+wall area). This value was calculated for 10 arterioles per patient. Data are presented as mean $\pm$ SD. Regression analysis, ANOVA, and  $t$  tests were performed with standard statistical software (SPSS). A value of  $P<.05$  was considered significant.

### Results

The HTx recipients, who served as control subjects, had no cardiac complaints, and all of them had normal coronary arteriograms. The time interval of catheterization after transplantation was  $4\pm 2$  years. Medication of HTx patients at the time of catheterization was immunosuppression ( $n=8$ )  $\text{Ca}^{2+}$  antagonists ( $n=8$ ), aspirin ( $n=5$ ), and dipyridamole ( $n=4$ ). No member of the control group had signs of rejection on the basis of the biopsies. Age distributions between the HCM ( $45.5\pm 14.6$  years) and control ( $48.7\pm 6.0$  years) groups were similar. HCM patients were symptomatic (NYHA class II or III), whereas all members of the control group were symptom free (NYHA class I). HCM patients had a subvalvular gradient of  $88\pm 31$  mm Hg and a lower aortic pressure ( $103\pm 14$  versus  $120\pm 15$  mm Hg,  $P<.05$ ), a higher end-diastolic left ventricular pressure ( $22\pm 1$  versus  $12\pm 6$  mm Hg,  $P<.05$ ), a lower cardiac index ( $2.7\pm 0.5$  versus  $3.5\pm 0.7$  L/m<sup>2</sup>,  $P<.05$ ), and a lower heart rate during baseline conditions ( $70\pm 13$  versus  $97\pm 13$  bpm,  $P<.05$ ) than the control group.

End-diastolic septal wall thickness was significantly increased in patients with HCM ( $25.8\pm 2.9$  mm) compared with members of the control group ( $11.4\pm 3.0$  mm;  $P<.05$ ). All HCM patients had normal angiograms. The diameter of the LAD was similar in both groups ( $3.0\pm 0.8$  versus  $3.0\pm 0.5$  mm). Coronary velocity during baseline conditions was higher for HCM patients ( $34\pm 11$  versus  $20\pm 11$  cm/s,  $P<.05$ ), whereas velocities during hyperemia were similar ( $49\pm 20$  versus  $53\pm 22$  cm/s). As a consequence, the CFR was reduced from  $2.6\pm 0.8$  in the control group to  $1.8\pm 0.9$  in the HCM group ( $P<.05$ ). Coronary resistance values, corrected for extravascular compression (see above), were lower ( $3.7\pm 2.1$  versus  $6.5\pm 2.2$  mm Hg  $\cdot$  s  $\cdot$  cm<sup>-1</sup>,  $P<.05$ ) during baseline conditions and were similar during hyperemia ( $2.6\pm 1.5$  versus  $2.6\pm 1.0$ ,  $P=\text{NS}$ ) in HCM. Consequently, the CRR was lower ( $1.5\pm 0.6$  versus  $2.6\pm 0.8$ ,  $P<.05$ ) in HCM than in the control group. Arteriolar wall area was similar ( $5720\pm 2130$  versus  $7107\pm 3544 \mu\text{m}^2$ ;  $P=\text{NS}$ ), but lumen area ( $1273\pm 688$  versus  $2260\pm 1165 \mu\text{m}^2$ ;  $P<.05$ ) and diameters were significantly lower ( $19.6\pm 4.5$  versus  $25.9\pm 4.3 \mu\text{m}$ ;  $P<.05$ ) in HCM compared with control values. Consequently, AL was lower in the HCM ( $21\pm 5\%$ ) than in the control group ( $30\pm 4\%$ ;  $P<.05$ ), and capillary density tended to decrease from  $1824\pm 424$  to  $1445\pm 513$  per square millimeter in HCM ( $P=.11$ ). In addition, both the CFR and the CRR were linearly related to AL according to the formula  $\text{CFR}=0.1 \text{AL}-0.45$  ( $r=.57$ ;  $P<.05$ ) and  $\text{CRR}=0.07 \text{AL}+0.35$  ( $r=.50$ ;  $P<.05$ ; Fig 1A). Further analysis revealed that the degree of AL ( $\text{AL}=-0.85 \text{Hyp}+43.7$ ;  $r=.71$ ;  $P<.05$ ; Fig 1B), the CFR ( $\text{CFR}=-0.17 \text{Hyp}+5.9$ ;  $r=.80$ ;  $P<.05$ ), the CRR ( $\text{CRR}=-1.2 \text{Hyp}+4.7$ ;  $r=.7$ ;  $P<.05$ ; Fig 1C), and the capillary density ( $\text{CD}=-51 \text{Hyp}+2750$ ;  $r=.53$ ;  $P<.05$ ; Fig 1D) were all inversely related to the degree of hypertrophy (Hyp). In addition, a linear relationship between AL and



Relationship of CRR with AL (A), AL and degree of hypertrophy (B), CRR and degree of hypertrophy (C), and capillary density and degree of hypertrophy (D).

capillary density was measured ( $AL=42 \text{ CD}+577$ ;  $r=.54$ ;  $P<.05$ ).

### Discussion

In symptomatic patients with HCM without evidence of a functional stenosis of the epicardial vessels, decrements in CFR were detected, confirming earlier studies.<sup>1-5</sup> Similar decrements in CRR were measured, implying that these findings could not be explained by increments in extravascular compression.<sup>9,10</sup> Abnormal arterioles with decreased lumen were detected in HCM patients, suggesting that a structural change in the coronary arterial vascular tree might be related to this finding. Indeed, a positive relationship between both CFR and CRR and AL, corrected for tissue shrinkage by normalization to the wall area,<sup>7</sup> was detected. Furthermore, an inverse relationship was noted between AL and the degree of hypertrophy, confirming earlier postmortem studies.<sup>7</sup> Because of this relationship, an inverse relationship between CFR and the degree of hypertrophy could be measured. Again, a similar relationship was found between CRR and degree of hypertrophy, implying that extravascular compressive forces were not essential for these findings. In large-animal models of pressure overload-

induced left ventricular hypertrophy, vascular medial hypertrophy has been observed only when the coronary circulation was exposed to high perfusion pressures. The present arteriolar abnormalities were obtained at normal to low aortic pressures and might imply that hypertrophy of the arterioles, in parallel to the hypertrophy of the myocardium, is an independent process.<sup>7</sup>

A decreased capillary density has been measured in several animal studies with experimentally induced secondary hypertrophy and recently in humans with secondary hypertrophy.<sup>12,13</sup> Although differences between the groups in capillary density did not reach levels of statistical significance, there clearly was an inverse relationship between capillary density and degree of hypertrophy. Furthermore, the decrements in capillary density and decrements of AL are related in HCM. These findings may imply that the decreased AL induces periods of ischemia, which results in increased angiogenesis. This angiogenesis normalizes the decrements in capillary density. However, because we did not analyze HCM myocardial tissue without hypertrophy, we cannot exclude the possibility that the occurrence of changes in the coronary microcirculation in HCM is a more independent phenomenon and not directly related to the degree of hypertrophy.<sup>6,7</sup>

In conclusion, septal hypertrophy is associated with decrements in CFR and CRR in HCM patients. Arterioles of HCM patients exhibited a smaller lumen at similar wall thickness, which correlated well with decrements in CFR and CRR. These findings suggest that abnormal arterioles might contribute to the perfusion abnormalities found in these patients, resulting in recurrent myocardial ischemia.

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