



Effects of myocardial fibrosis assessed by magnetic resonance imaging on dynamic left ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2012-001267
Article Type:	Research
Date Submitted by the Author:	19-Jul-2012
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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Cardiomyopathy < CARDIOLOGY, Echocardiography < CARDIOLOGY, Adult cardiology < CARDIOLOGY

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Manuscripts

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3 **Effects of myocardial fibrosis assessed by magnetic resonance imaging on dynamic left**
4 **ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy: a**
5 **retrospective database analysis.**
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Abstract:

Background: While implications of myocardial fibrosis on left ventricular (LV) function at rest have been studied in hypertrophic cardiomyopathy (HCM), the pathophysiological consequences on dynamic LV outflow tract (LVOT) gradient have so far not been investigated in detail.

Objective: To evaluate the influence of myocardial fibrosis, detected by magnetic resonance imaging as late gadolinium enhancement (LGE), on LVOT gradient in HCM.

Design: Retrospective database analysis.

Setting: A single Italian cardiomyopathies referral center.

Patients: Seventy-six HCM patients with normal ejection fraction at rest.

Interventions: Patients underwent cardiac magnetic resonance and performed bicycle exercise echocardiogram within a month.

Results: LGE was present in 54 patients (71%), ranging from 0,2% to 32,4% of left ventricular mass. There was a weak correlation between the amount of fibrosis and LVOT gradient variation during exercise in the overall population ($r = -0,243$, $p = 0,034$) and a stronger correlation in patients with obstructive HCM at rest ($r = -0,524$, $p = 0,021$). Patients with an LVOT gradient increase ≥ 50 mmHg during exercise had a significantly lesser extent of fibrosis than those with an increase < 50 mmHg (0,7% (IQR 0-2,4) vs 3,2% (IQR 0.2-7,4), $p = 0,006$). The extent of fibrosis was significantly lower among the highest quartiles of LVOT gradient increase ($p = 0,009$).

Conclusions: In patients with HCM and normal ejection fraction at rest, myocardial fibrosis was associated with a lower increase in LVOT gradient during exercise, probably due to a lesser



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3 degree of myocardial contractility recruitment. This negative association was more evident in
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5 patients with an obstructive form at rest.
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10 **BACKGROUND**

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12 The pathophysiology of hypertrophic cardiomyopathy (HCM) is the result of a number of
13 interrelated factors that include impaired ventricular relaxation, increased myocardial stiffness,
14 myocardial ischemia, left ventricular outflow tract (LVOT) obstruction and mitral regurgitation
15 [1, 2]. In recent years magnetic resonance imaging (MRI) and exercise echocardiography have
16 opened new possibilities for non-invasive evaluation of myocardial substrate and LV function in
17 HCM [3-8]. In particular, gadolinium-enhanced MRI has shown that a high percentage of
18 patients with HCM has variable degrees of late gadolinium enhancement (LGE) which, in this
19 disease, has been shown to correspond to interstitial fibrosis [6-9]. Myocardial fibrosis in HCM
20 has been shown to be associated with an increased incidence of sudden death risk factors
21 —particularly ventricular arrhythmias— [9-11] and an increased risk of HCM-related morbidity
22 and mortality [12-14]. Additionally, myocardial fibrosis influences LV function at rest
23 negatively [15-16]. Not only are large confluent areas of LGE associated with the end-stage
24 phase of the disease, but also lesser amounts of fibrosis seem to determine a reduction in LV
25 systolic function at rest (albeit within the “normal” ejection fraction (EF) range) [15]. The
26 relationship between myocardial fibrosis and LV function during exercise remains unexplored.
27 Since LVOT gradient during exercise is mainly related to the increase in LV contractility, we
28 aimed to explore the relationship between MRI assessed myocardial fibrosis and LV outflow
29 gradient during exercise echocardiography in HCM patients with normal LV EF.
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METHODS

Patients

Ninety-one consecutive outpatients, evaluated at the S. Orsola-Malpighi University Hospital Bologna, Italy between January 2009 and November 2010, were considered for the study. Fifteen patients were excluded due to coexistent coronary artery disease (4 patients), atrial fibrillation (n=3), previous surgical septal myectomy (n=1), LV EF <50% at rest (n=2) and general contraindications / refusal to MRI (n=5). All patients underwent contrast-enhanced cardiac MRI and bicycle exercise echocardiogram within a 1 month period. All patients fulfilled conventional criteria for HCM with LV hypertrophy ≥ 15 mm [17].

All patients provided written informed consent for exercise echocardiography and magnetic resonance. No specific ethical approval was required for this study that included only non-invasive examinations that HCM patients routinely undergo at our institution.

Exercise echocardiography protocol

Having suspended beta-blockers and/or calcium antagonist and dysopyramide for at least 5 half-lives, patients performed a symptom-limited bicycle exercise stress test in semi supine position on an exercise echo-tilting table (stress echo supine ergometer, Ergoselect 1200 EL, Ergoline GmbH, Bitz, Germany). The workload was increased by 25 watts every 2 minutes. Blood pressure and 12-lead ECG were recorded every minute. Echocardiographic images were assessed at baseline and at peak exercise using a Philips Sonos 5500 Ultrasound System (Philips Ultrasound, Andover, MA, USA) equipped with a harmonic fusion imaging probe (s3) and off-line cine loop analysis software. All images were recorded digitally and analysed off-line and each parameter was measured on an average of three consecutive beats both at rest and during



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3 exercise. LV volumes and EF were calculated using the Simpson method from the apical 4 and
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5 2-chamber view. LV volumes were normalized to the body surface area. Mitral regurgitation was
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7 quantified with the colour-area method. Continuous wave Doppler was used to measure LV
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9 outflow gradient from the apical 4-chamber view. The early filling (E) and late (A) filling
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11 velocities, as well as the deceleration time (DT) of early filling, were measured from the
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13 transmitral flow. Tissue Doppler velocities were recorded from the medial (septal) and lateral
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15 mitral annulus as previously reported [18] and averaged; the ratio of early mitral diastolic inflow
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17 velocity to early diastolic mitral annular velocity (E/E') was calculated.
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25 **MRI technique**

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27 MRI was performed on a 1.5 T scanner (Signa Twin Speed Excite, General Electric, Milwaukee,
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29 WI, USA) with surface coils and prospective ECG triggering. LV end-systolic and end-diastolic
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31 diameters as well as maximal (end-diastolic) wall thickness were traced and recorded from the
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33 short axis and long axis views (8 mm slice thickness, no gap) of the standard ECG-gated steady
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35 state free precession (SSFP) cine sequence. Image parameters were: repetition time of 3.5 msec,
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37 echo time 1.6 msec, temporal resolution 40 msec, matrix 224 x 160, flip angle 45°, bandwidth
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39 125 kHz, views per segment 8 to 16. LV volumes, mass and EF were measured from a stack of
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41 sequential 8 mm short axis slices (no gap) from the atrio-ventricular ring to the apex, through
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43 analysis with a commercially available software (Mass Analysis Plus, Medis, Leiden, The
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45 Netherlands) and were indexed to body surface in m². LGE images for detection of delayed
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47 hyper-enhancement were acquired 10-15 minutes after intravenous administration of
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49 Gadopentate dimeglumine (0.2 mmol/kg) (Magnevist; Schering, Berlin, Germany) using a
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51 breath-hold segmented inversion recovery fast gradient echo sequence in the short axis and in
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3 long axis planes of the LV, with 9 mm slice thickness and no gap. Image parameters were:
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5 repetition time of 5.3 ms, echo time 1.3 ms, flip angle 20°, matrix 256 x 160, NEX 2 and field of
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7 view 320 mm. Optimal inversion time to null normal myocardial signal was determined for each
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9 patient and ranged from 220 to 320 ms. After visual inspection of all short axis LV slices to
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11 identify areas of completely nulled myocardium (normal myocardium), the mean signal intensity
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13 of normal myocardial tissue was calculated and a threshold ≥ 2 standard deviations exceeding the
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15 mean was used to identify LGE areas. This limit was deemed acceptable to discriminate LGE
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17 from healthy myocardium without reducing sensibility. LGE areas were outlined manually and
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19 the total volume (expressed in grams) was quantified using a specific software (ReportCard, GE
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21 Medical Systems, Milwaukee, WI, USA) and expressed as percentage of LV mass. LGE analysis
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23 was performed by one experienced reader (L.L., > 8 years of MRI experience) and reviewed by a
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25 second reader (R.F., > 10 years of MRI experience).
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34 **Study design and statistical analysis**

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36 In order to explore a possible relation between myocardial fibrosis and LV outflow obstruction
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38 during exercise the following analyses were planned:
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41 • Linear regression analysis between the extent of fibrosis and maximum LVOT gradient
42 during exercise;
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44 • Linear regression analysis between the extent of fibrosis and changes in LVOT gradient
45 during exercise (in the overall population and in the patients with an obstructive form at rest,
46 defined as LV gradient ≥ 30 mmHg);
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48 • Comparison of fibrosis extent between patients with maximum gradient \geq or $<$ 50 mmHg,
49 and between patients with an increase in exercise gradient \geq or $<$ 50 mmHg,
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- Comparison of fibrosis extent between patients with a gradient increase above or below the median value in our population, and among different quartiles of gradient increase.

Categoric variables are expressed as total numbers and percentages. Continuous variables are expressed as median values (interquartile range, IQR). Comparison of categoric variables was performed with the chi-square test and continuous variables were analyzed with the Mann-Whitney U test, Wilcoxon signed-rank test and Kruskal-Wallis test as appropriate. A p value of 0.05 was considered to be statistically significant. Regarding echocardiographic measurements, intra-observer variability was assessed in two different blind evaluations 30 days apart, whereas inter-observer variability was assessed by two different observers (G.R. and E.B.). Both assessments were made on a 15 patient sample. Data processing and statistical analyses were performed using the SPSS 15.0 statistical program (SPSS Inc., Chicago, IL, USA).

RESULTS

LGE was present in 54 patients (71%), involving a percentage of LV mass ranging from 0,2% to 32,4%. Fibrosis consisted of small, diffuse areas in 32 patients (59%) and was confluent into a smaller number of larger areas in 22 patients (41%). Table 1 reports the clinical, resting echocardiographic and MRI characteristics of the study population. Regarding echocardiographic measurements, mean intra-observer variability for end-diastolic and for end-systolic volume were 4 ± 1 ml/m² and 3 ± 1 ml/m² respectively. Mean inter-observer variability for end-diastolic and for end-systolic volume were 5 ± 1 ml/m² and 4 ± 1 ml/m² respectively. Mean intra-observer and inter-observer variability of Doppler indexes of LV filling were as follows: E wave, 0.08 ± 2.36 cm/sec and 1.20 ± 4.30 cm/sec; A wave, 0.12 ± 1.96 cm/sec and 0.64 ± 4.54 cm/sec.



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The variation of echocardiographic characteristics from rest to exercise is reported in Table 2. HCM patients performed a maximum workload of 100 W (IQR 75-125) with a median heart rate increase from 73 (IQR 66-84) bpm to 128 (IQR 112-142) bpm. Median LV outflow gradient increased from 11 (IQR 7-31) mmHg to 27 (IQR 16-98) mmHg on exercise; 15 patients (20%) without obstruction at rest developed a gradient ≥ 30 mmHg on exercise and 18 (24%) had an increase in outflow gradient ≥ 50 mmHg. In 28 patients (37%) EF did not increase or decreased with exercise.

There was no correlation between the extent of fibrosis and maximum LVOT gradient during exercise ($r = -0,197$, $p = 0,087$). Considering the variation in LVOT gradient during exercise, there was a weak correlation with the extent of fibrosis in the overall population ($r = -0,243$, $p = 0,034$) and a stronger correlation in patients with an obstructive form of the disease at rest ($r = -0,524$, $p = 0,021$), (Figure 1).

Patients with a maximum gradient during exercise ≥ 50 mmHg tended to have a lesser amount of fibrosis than those with a maximum gradient < 50 mmHg, the difference however did not reach significance (1,1% (IQR 0-3,9) vs 4,1% (IQR 0,5-8,2), $p = 0,089$). Patients with an increase in LVOT gradient ≥ 50 mmHg had a significantly lesser extent of fibrosis than those with an increase < 50 mmHg (0,7% (IQR 0-2,4) vs 3,2% (IQR 0,2-7,4), $p = 0,006$) (Figure 2). There was no difference in terms of fibrosis extent between patients with an increase in LVOT gradient \geq or $<$ than the median value (14 mmHg) (1,7% (IQR 0-4,6) vs 2,8% (IQR 0-7,0), $p = 0,330$). When dividing the population in to quartiles according to LVOT gradient increase during exercise the extent of fibrosis was significantly different: in patients with an increase < 8 mmHg the median value of fibrosis was 3,4% (IQR 2,2-8,6), in patients with an increase between 8 and 13 mmHg was 1,1% (IQR 0,0-6,6), in patients with an increase between 14 and 46 mmHg was 4,4% (IQR



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3 0,7-11,6) and in patients with an increase ≥ 47 mmHg median value of fibrosis was 0,6% (IQR
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5 0,0-2,4) ($p=0,009$).
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10 **DISCUSSION**

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12 This study shows that myocardial fibrosis (detected as LGE on MRI) may influence the
13 development of LVOT gradient during exercise in patients with HCM and normal EF: patients
14 with higher exercise-induced gradients show a lesser degree of myocardial fibrosis and vice
15 versa (Figure 3). This negative association is more evident in patients with an obstructive form at
16 rest.
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24 In recent years, myocardial fibrosis has been emerging as an important actor in the complex
25 pathophysiology of HCM. It has been suggested that impairment in collagen turnover could be a
26 component of the disease phenotype and that it appears as an early manifestation of sarcomere
27 gene mutations, before the development of overt LV hypertrophy [19-20]. When hypertrophy
28 develops, increasing amounts of interstitial fibrosis can be detected noninvasively by
29 gadolinium-enhanced cardiac MRI [6-8]. The exact mechanism leading to fibrosis remains
30 unknown but it has been hypothesized that the main triggers for the fibrotic process include
31 molecular factors at cellular level (induced by sarcomeric mutations), hemodynamic factors
32 (overall ventricular afterload resulting from the sum of LV outflow obstruction and systolic
33 blood pressure), and ischemia (mainly related to small intramural coronary vessel disease) [8].
34 Myocardial fibrosis in HCM has been associated with the risk of life-threatening arrhythmias and
35 with a wide spectrum of systolic dysfunction, ranging from a mild LV EF reduction to the end
36 stage phase [9-14]. The present study confirmed the association between myocardial fibrosis and
37 contractility, assuming that LV systolic function is one of the major determinants of the LVOT
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gradient increase during effort. The prevalence of myocardial fibrosis (71%) in our study is comparable with that of the largest published series [12, 14], in most cases however, LGE was modest and presented a patchy distribution. Our results therefore support the concept of a continuum of hemodynamic effect of myocardial fibrosis on LV function. Large "scar-like" areas of fibrosis are a determinant of the end-stage evolution, lesser degrees of fibrosis are associated with slight EF reduction [14, 15], while even lesser degrees of fibrosis, while not influencing EF at rest, seem to result in a lesser contractility recruitment during exercise, leading to a lower LV outflow gradient. Notably, the effects of myocardial fibrosis were particularly evident among patients with LV outflow gradient already present at rest. Indeed LV contractility is not the only determinant of LV outflow obstruction; excessive length of the anterior mitral leaflet, abnormalities in the subvalvular apparatus and load conditions also play a role [21]. In patients with no LV outflow obstruction at rest (related for example to the large anatomical size of LVOT and/or a non-redundant mitral valve), the increase in contractility could fail to generate a significant LV gradient increase regardless of the amount of myocardial fibrosis.

Study limitations

The main limitations of this hypothesis generating study are related to the low number of patients. The lack of direct hemodynamic measurement of LV pressures limits the pathophysiological interpretation of our data which is essentially based on the behavior of LV outflow gradient and indexes of ventricular and myocardial function. Also, our study did not include a detailed analysis of the behaviour of LV volumes during exercise and of their correlation with other variables. Indeed, the small absolute values of LV end-systolic volume in



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3 this disease during exercise (often below the repeatability threshold) make echocardiography an
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5 unreliable technique for this purpose.
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10 **CONCLUSIONS**

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12 In patients with HCM and normal EF at rest, myocardial fibrosis —detected by MRI— is
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14 associated with a lower increase in LVOT gradient during exercise, probably due to a lesser
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16 degree of myocardial contractility recruitment. This negative association is more evident in
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18 patients with an obstructive form at rest.
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24 **COMPETING INTERESTS**

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27 The authors have no competing interests to declare.
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32 **CONTRIBUTIONS**

33
34 GR, LL, FL, SR, CP, FP, MLBR, RF: substantial contributions to conception and design,
35
36 acquisition of data, or analysis and interpretation of data; EB, ML, IO: drafting the article or
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38 critical revision; and CR: final approval of the manuscript
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44 **FUNDING.**

45
46 This research received no specific grant from any funding agency in the public, commercial or
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48 not-for-profit sectors.
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Table 1. Baseline clinical, echocardiographic and magnetic resonance characteristics.

<i>Clinical</i>	
No. of patients, n (%)	76
Males, n (%)	51 (67%)
Age, years	48 (41-61)
Family history of HC, n (%)	34 (45%)
Family history of SD, n (%)	10 (13%)
NYHA functional class I, n (%)	61 (80%)
II, n (%)	14 (18%)
Unexplained syncope, n (%)	12 (16%)
NSVT on Holter monitor, n (%)	21 (28%)
<i>Echocardiography:</i>	
LV gradient ≥ 30 mmHg at rest, n (%)	20 (26%)
Maximum WT, mm	20 (17-23)
Maximum WT ≥ 30 mm, n (%)	3 (4%)
Left atrium diameter, mm	43 (39-48)
<i>Magnetic resonance imaging:</i>	
LV mass, g/m ²	155 (124-196)
LV mass/end-diastolic volume, g/ml	1.09 (0.92-1.46)
LGE % of LV mass, (%)	2.4 (0-6)



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3 Legend: HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LV left
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5 ventricle; NSVT, non-sustained ventricular tachycardia; NYHA: New York Heart Association;
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8 SD: sudden death; WT: wall thickness.
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10 Continuous variables are expressed as median values (interquartile range, IQR).
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Table 2. Echocardiographic data at rest and during exercise.

	Rest	Exercise	p value
Maximum workload, W		100 (75, 125)	
Heart rate, bpm	73 (66, 84)	128 (112, 142)	<0.001
Left ventricle outflow gradient, mmHg	11 (7, 31)	27 (16, 98)	<0.001
Δ Left ventricle outflow gradient, mmHg		14 (8, 46)	
Mitral regurgitation jet area, cm ²	1.2 (0.1, 3.1)	3.0 (0.6, 7.1)	<0.001
Δ Mitral regurgitation jet area, cm ²		0.6 (0, 3.6)	
End-diastolic volume, ml/m ²	35 (28, 45)	29 (20, 40)	<0.001
Δ End-diastolic volume, ml/m ²		-6 (-12, -2)	
End-systolic volume, ml/m ²	8 (5, 12)	5 (3, 7)	<0.001
Δ End-systolic volume, ml/m ²		-3 (-5, 0)	
Stroke volume, ml/m ²	28 (22, 37)	23 (17, 32)	<0.001
Δ Stroke volume, ml/m ²		-4 (-9, 1)	
Ejection fraction, %	78 (71, 84)	83 (75, 88)	<0.001
Δ Ejection fraction, %		5 (-2, 11)	
E wave, cm/s	71 (59, 89)	97 (83, 121)	<0.001
Δ E wave, cm/s		25 (8, 44)	
A wave, cm/s	73 (60, 91)	104 (84, 125)	<0.001
Δ A wave, cm/s		27 (8, 45)	
Deceleration time, ms	185 (160, 250)	N.A.	/
S wave, cm/s	7.5 (6.1, 9.0)	9.4 (7.6, 11.9)	<0.001
Δ S wave, cm/s		2.1 (0.7, 3.3)	
E' wave, cm/s	7.5 (5.9, 9.0)	9.7 (7.4, 13.8)	<0.001
Δ E' wave, cm/s		3.1 (0.9, 5.0)	
A' wave, cm/s	8.4 (6.5, 11.1)	11.1 (9.3, 15.5)	<0.001
Δ A' wave, cm/s		2.5 (0.9, 5.1)	
E/E'	9.9 (7.0, 14.2)	9.2 (7.1, 12.8)	0.270



Figure legends

Figure 1. Linear regression analysis between extent of fibrosis and changes in LV outflow tract gradient during exercise. A: in the overall population, B: in patients with obstructive HCM at rest.

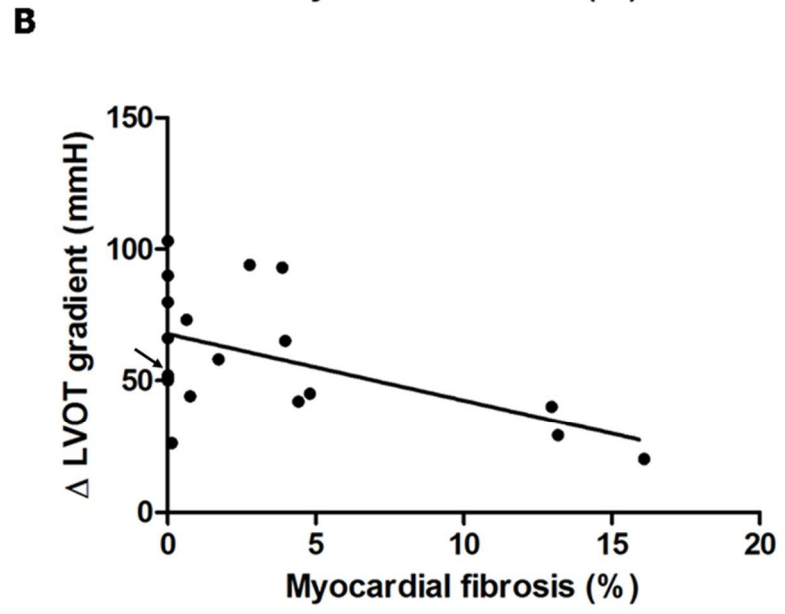
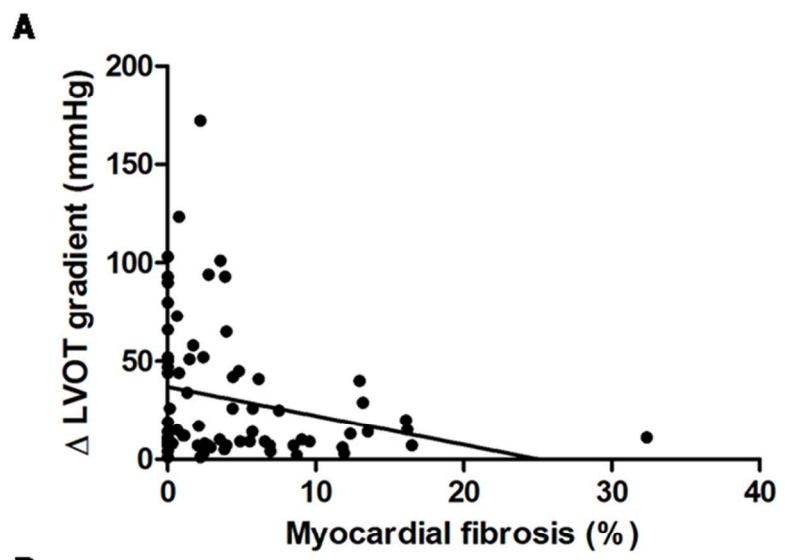
Note: the mark indicated by the arrow represents four patients that showed no LGE and an increase in LVOT gradient during exercise of 50, 50, 51 and 52 mmHg respectively.

Figure 2. Fibrosis extent (expressed as median and interquartile range) in patients with an increase in exercise gradient $<$ or \geq 50 mmHg.

Figure 3. Myocardial fibrosis and changes in LV outflow tract gradient during exercise. A: patient with a large amount of myocardial fibrosis and modest increase in LV outflow tract gradient. B: patient with a limited amount of fibrosis and relevant increase in LV outflow tract gradient during exercise.

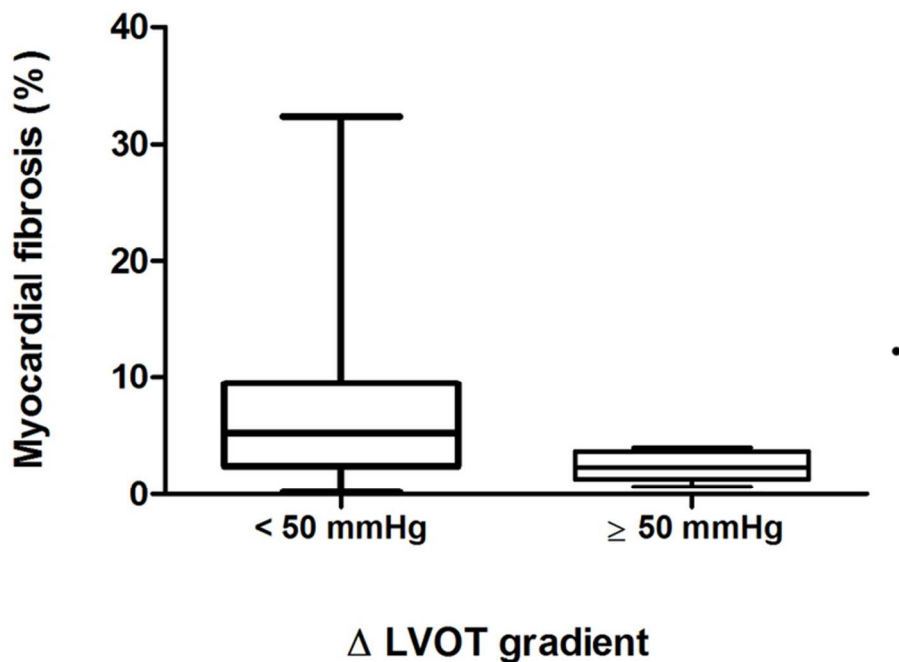


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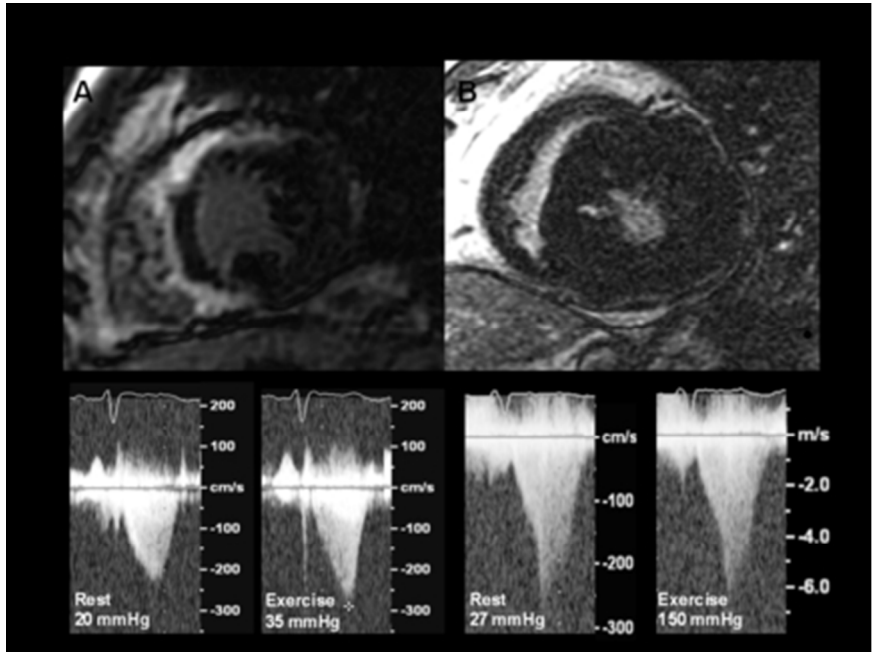
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3 Reviewer Comments:
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6 **Reviewer: 1**

7 *The paper is well written and the echo and CMR methods are robust. The statistical methods are*
8 *sound though some caution has to be given to the multiple subgroups used to find statistically*
9 *significant relationships. My main question for the authors is what do the results mean to*
10 *readers of quite a general cardiology journal. When you look at the raw scatter plots and*
11 *R values the relationship is really quite weak so I am unsure how this will influence clinical*
12 *practice or guide further studies and I think this really needs to be elaborated on in the*
13 *discussion.*

14
15 **Thank you for the observations.**

16 **Our study was designed to be small and hypothesis generating and we do not think that our**
17 **results should influence current clinical practice. However, myocardial fibrosis in HCM is**
18 **currently the object of a considerable amount of research regarding it's pathophysiology**
19 **and meaning. Our results should be read along with these other papers as a basis for**
20 **expanding the knowledge of HCM and designing larger studies combining cardiac MRI**
21 **and exercise echocardiography.**

22
23
24
25 *The authors describe reproducibility measurements in the methods but need to*
26 *give the results of these as it may be the errors here are larger than those*
27 *between the different groups.*

28
29 **Thank you for the observation, we have now added this data (page 6, lines 15-20).**

30
31
32 Reviewer: 2
33

34
35 1. Page 5: The authors lay out on Page 5 four points for study to examine
36 the association between fibrosis and gradient provocation. Other factors that
37 would be important to consider are whether the severity and duration of
38 exposure to excessive ventricular load could cause the fibrosis detected by
39 gadolinium enhancement. This effect could also potentially be at least
40 partially diminished in some of the patients by early exposure to gradient
41 suppressing agents (e.g., beta-blockade, verapamil, disopyramide, etcetera).
42 Additionally, the load experienced by the myocardium would be proportional to
43 the gradient plus the peak systolic blood pressure. Finally, the severity of
44 left ventricular wall thickness may also play a role in determining the
45 presence of both fibrosis formation and gradient formation.
46
47

48 **Thank you for the observations. We agree that the relationship between fibrosis and**
49 **outflow gradient is complex and includes many interrelated factors. We have therefore**
50 **modified the discussion section of our paper in order to include these concepts. The revised**
51 **version reads as follows: "The exact mechanism leading to fibrosis remains unknown but it**
52 **has been hypothesized that the main triggers for the fibrotic process include molecular**
53 **factors at cellular level (induced by sarcomeric mutations), hemodynamic factors (overall**
54 **ventricular afterload resulting from the sum of LV outflow obstruction and systolic blood**
55 **pressure), and ischemia (mainly related to small intramural coronary vessel disease) [8]."**
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4 2. Page 6, Results: Can the authors define the difference between diffuse
5 and confluent gadolinium enhancement patterns? Is this reproducible by
6 others?
7

8 **The categorization was carried out subjectively by experienced cardiac MRI readers, as in**
9 **most published papers on this topic. In order to clarify our definition however, we have**
10 **modified the manuscript as follows: “Fibrosis consisted of small, diffuse areas in 32**
11 **patients (59%) and was confluent into a smaller number of larger areas in 22 patients**
12 **(41%)”. The two pattern used to describe late Gd enhancement were included solely for**
13 **descriptive purposes as they were not used in any of our analyses.**
14

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16
17 3. Page 7: The authors describe quartiles of gradient increase as groups in
18 which they assessed extent of fibrosis. These results do not show a clear dose
19 response in terms of fibrosis dose leading to lower gradient response.
20

21
22 **The small number of patients included in our study probably underpowers it statistically**
23 **and is probably responsible for the absence of a clear dose response in the analysis of the**
24 **population divided into quartiles according to gradient increase. However, the fact that the**
25 **extent of fibrosis was significantly lower in the highest quartile of LVOT gradient increase**
26 **and higher in the lowest quartile of LVOT gradient increase indicates a trend and is in line**
27 **with our other findings. As stated in the limitations section, this was conceived as a**
28 **hypothesis generating study and larger studies are necessary to confirm our hypotheses.**
29
30

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32
33 4. Page 7, last paragraph: The authors state that fibrosis “can influence
34 the development of LVOT gradient”. What the authors have shown is an
35 association between the two entities and not a cause and effect relationship.
36

37
38 **Thank you for the observation. You are correct in saying that we have only shown an**
39 **association between to entities and we hypothesize that this is the result of a cause and**
40 **effect relationship. We have therefore modified our manuscript as follows: “This study**
41 **shows that myocardial fibrosis (detected as LGE on MRI) may influence the development**
42 **of LVOT gradient during exercise in patients with HCM and normal EF: patients with**
43 **higher exercise-induced gradients show a lesser degree of myocardial fibrosis and vice**
44 **versa (Figure 3).”**
45

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48
49 5. Page 8: Again, the authors have stated they have investigated the “role”
50 of fibrosis in gradient development. Again, they have shown a statistical
51 association between the two entities.
52

53
54 **As above you are correct, thank you. We have therefore modified the manuscript that now**
55 **reads as follows: “The present study confirmed the association between myocardial fibrosis**
56 **and contractility, assuming that LV systolic function is one of the major determinants of**
57 **the LVOT gradient increase during effort.”**
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4 6. Figure 1B: The linear regression plot of myocardial fibrosis versus
5 delta LVOT gradient is instructive in that it clearly shows that three patients
6 are the primary determinants of this regression curve (those three patients
7 with nearly 15% of the myocardium replaced by fibrosis). Similarly, these
8 three patients have a delta LVOT gradient that overlaps with patients who have
9 almost no change in LVOT gradient with exercise.
10

11
12 **We agree with this observation. Given the small number of patients the statistical**
13 **significance emerges due to patients with an extreme behaviour. However, this does not**
14 **undermine the statistical significance of the study or its potential hypothesis generating**
15 **role.**
16

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20 7. Was the delta gradient in any way related to the resting gradient? In
21 other words, were patients with resting gradient more likely to have a higher
22 or lower change in gradient with exercise?
23

24
25 **Thank you for the observation. The data was not included in our paper but patients with a**
26 **significant obstruction at rest do tend to develop a greater gradient during exercise. In**
27 **order to not complicate the paper we would prefer to not include this data in the**
28 **manuscript.**
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STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Effects of myocardial fibrosis assessed by magnetic resonance imaging on dynamic left ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2012-001267.R1
Article Type:	Research
Date Submitted by the Author:	03-Sep-2012
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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Radiology and imaging
Keywords:	Cardiomyopathy < CARDIOLOGY, Echocardiography < CARDIOLOGY, Adult cardiology < CARDIOLOGY

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Manuscripts

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3 **Effects of myocardial fibrosis assessed by magnetic resonance imaging on dynamic left**
4 **ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy: a**
5 **retrospective database analysis.**
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12 Elena Biagini¹, Massimiliano Lorenzini¹, Iacopo Olivetto³, Guido Rocchi¹, Luigi Lovato²,
13 Francesco Lai¹, Stefania Rosmini¹, Chiara Pazzi¹, Ferdinando Pasquale¹, Maria Letizia Bacchi
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BACKGROUND

The pathophysiology of hypertrophic cardiomyopathy (HCM) is the result of a number of interrelated factors that include impaired ventricular relaxation, increased myocardial stiffness, myocardial ischemia, left ventricular outflow tract (LVOT) obstruction and mitral regurgitation [1, 2]. In recent years magnetic resonance imaging (MRI) and exercise echocardiography have opened new possibilities for non-invasive evaluation of myocardial substrate and LV function in HCM [3-8]. In particular, gadolinium-enhanced MRI has shown that a high percentage of patients with HCM has variable degrees of late gadolinium enhancement (LGE) which, in this disease, has been shown to correspond to interstitial fibrosis [6-9]. Myocardial fibrosis in HCM has been shown to be associated with an increased incidence of sudden death risk factors—particularly ventricular arrhythmias— [9-11] and an increased risk of HCM-related morbidity and mortality [12-14]. Additionally, myocardial fibrosis influences LV function at rest negatively [15-16]. Not only are large confluent areas of LGE associated with the end-stage phase of the disease, but also lesser amounts of fibrosis seem to determine a reduction in LV systolic function at rest (albeit within the “normal” ejection fraction (EF) range) [15]. The relationship between myocardial fibrosis and LV function during exercise remains unexplored. Since LVOT gradient during exercise is mainly related to the increase in LV contractility, we designed a hypothesis generating study to explore the relationship between MRI assessed myocardial fibrosis and LV outflow gradient during exercise echocardiography in HCM patients with normal LV EF.

METHODS

Patients



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3 Ninety-one consecutive outpatients, evaluated at the S. Orsola-Malpighi University Hospital
4 Bologna, Italy between January 2009 and November 2010, were considered for the study. Fifteen
5
6 patients were excluded due to coexistent coronary artery disease (4 patients), atrial fibrillation
7
8 (n=3), previous surgical septal myectomy (n=1), LV EF <50% at rest (n=2) and general
9
10 contraindications / refusal to MRI (n=5). All patients underwent contrast-enhanced cardiac MRI
11
12 and bicycle exercise echocardiogram within a 1 month period. All patients fulfilled conventional
13
14 criteria for HCM with LV hypertrophy ≥ 15 mm [17].

15
16 All patients provided written informed consent for exercise echocardiography and magnetic
17
18 resonance. No specific ethical approval was required for this study that included only non-
19
20 invasive examinations that HCM patients routinely undergo at our institution.
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30 **Exercise echocardiography protocol**

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32 Having suspended beta-blockers and/or calcium antagonist and dysopiramide for at least 5 half-
33
34 lives, patients performed a symptom-limited bicycle exercise stress test in semi supine position
35
36 on an exercise echo-tilting table (stress echo supine ergometer, Ergoselect 1200 EL, Ergoline
37
38 GmbH, Bitz, Germany). The workload was increased by 25 watts every 2 minutes. Blood
39
40 pressure and 12-lead ECG were recorded every minute. Echocardiographic images were assessed
41
42 at baseline and at peak exercise using a Philips Sonos 5500 Ultrasound System (Philips
43
44 Ultrasound, Andover, MA, USA) equipped with a harmonic fusion imaging probe (s3) and off-
45
46 line cineloop analysis software. All images were recorded digitally and analysed off-line and
47
48 each parameter was measured on an average of three consecutive beats both at rest and during
49
50 exercise. LV volumes and EF were calculated using the Simpson method from the apical 4 and
51
52 2-chamber view. LV volumes were normalized to the body surface area. Mitral regurgitation was
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quantified with the colour-area method. Continuous wave Doppler was used to measure LV outflow gradient from the apical 4-chamber view. The early filling (E) and late (A) filling velocities, as well as the deceleration time (DT) of early filling, were measured from the transmitral flow. Tissue Doppler velocities were recorded from the medial (septal) and lateral mitral annulus as previously reported [18] and averaged; the ratio of early mitral diastolic inflow velocity to early diastolic mitral annular velocity (E/E') was calculated.

MRI technique

MRI was performed on a 1.5 T scanner (Signa Twin Speed Excite, General Electric, Milwaukee, WI, USA) with surface coils and prospective ECG triggering. LV end-systolic and end-diastolic diameters as well as maximal (end-diastolic) wall thickness were traced and recorded from the short axis and long axis views (8 mm slice thickness, no gap) of the standard ECG-gated steady state free precession (SSFP) cine sequence. Image parameters were: repetition time of 3.5 msec, echo time 1.6 msec, temporal resolution 40 msec, matrix 224 x 160, flip angle 45°, bandwidth 125 kHz, views per segment 8 to 16. LV volumes, mass and EF were measured from a stack of sequential 8 mm short axis slices (no gap) from the atrio-ventricular ring to the apex, through analysis with a commercially available software (Mass Analysis Plus, Medis, Leiden, The Netherlands) and were indexed to body surface in m². LGE images for detection of delayed hyper-enhancement were acquired 10-15 minutes after intravenous administration of Gadopentate dimeglumine (0.2 mmol/kg) (Magnevist; Schering, Berlin, Germany) using a breath-hold segmented inversion recovery fast gradient echo sequence in the short axis and in long axis planes of the LV, with 9 mm slice thickness and no gap. Image parameters were: repetition time of 5.3 ms, echo time 1.3 ms, flip angle 20°, matrix 256 x 160, NEX 2 and field of



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2
3 view 320 mm. Optimal inversion time to null normal myocardial signal was determined for each
4
5 patient and ranged from 220 to 320 ms. After visual inspection of all short axis LV slices to
6
7 identify areas of completely nulled myocardium (normal myocardium), the mean signal intensity
8
9 of normal myocardial tissue was calculated and a threshold ≥ 2 standard deviations exceeding the
10
11 mean was used to identify LGE areas. This limit was deemed acceptable to discriminate LGE
12
13 from healthy myocardium without reducing sensibility. LGE areas were outlined manually and
14
15 the total volume (expressed in grams) was quantified using a specific software (ReportCard, GE
16
17 Medical Systems, Milwaukee, WI, USA) and expressed as percentage of LV mass. LGE analysis
18
19 was performed by one experienced reader (L.L., > 8 years of MRI experience) and reviewed by a
20
21 second reader (R.F., > 10 years of MRI experience).
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29 **Study design and statistical analysis**

30
31 In order to explore a possible relation between myocardial fibrosis and LV outflow obstruction
32
33 during exercise the following analyses were planned:
34

- 35
36 • Linear regression analysis between the extent of fibrosis and maximum LVOT gradient
37
38 during exercise;
- 39
40 • Linear regression analysis between the extent of fibrosis and changes in LVOT gradient
41
42 during exercise (in the overall population and in the patients with an obstructive form at rest,
43
44 defined as LV gradient ≥ 30 mmHg);
- 45
46 • Comparison of fibrosis extent between patients with maximum gradient \geq or $<$ 50 mmHg,
47
48 and between patients with an increase in exercise gradient \geq or $<$ 50 mmHg,
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- 51
52 • Comparison of fibrosis extent between patients with a gradient increase above or below the
53
54 median value in our population, and among different quartiles of gradient increase.
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Categoric variables are expressed as total numbers and percentages. Continuous variables are expressed as median values (interquartile range, IQR). Comparison of categoric variables was performed with the chi-square test and continuous variables were analyzed with the Mann-Whitney U test, Wilcoxon signed-rank test and Kruskal-Wallis test as appropriate. A p value of 0.05 was considered to be statistically significant. Regarding echocardiographic measurements, intra-observer variability was assessed in two different blind evaluations 30 days apart, whereas inter-observer variability was assessed by two different observers (G.R. and E.B.). Both assessments were made on a 15 patient sample. Data processing and statistical analyses were performed using the SPSS 15.0 statistical program (SPSS Inc., Chicago, IL, USA).

RESULTS

LGE was present in 54 patients (71%), involving a percentage of LV mass ranging from 0,2% to 32,4%. Fibrosis consisted of small, diffuse areas in 32 patients (59%) and was confluent into a smaller number of larger areas in 22 patients (41%). Table 1 reports the clinical, resting echocardiographic and MRI characteristics of the study population. Regarding echocardiographic measurements, mean intra-observer variability for end-diastolic and for end-systolic volume were 4 ± 1 ml/m² and 3 ± 1 ml/m² respectively. Mean inter-observer variability for end-diastolic and for end-systolic volume were 5 ± 1 ml/m² and 4 ± 1 ml/m² respectively. Mean intra-observer and inter-observer variability of Doppler indexes of LV filling were as follows: E wave, 0.08 ± 2.36 cm/sec and 1.20 ± 4.30 cm/sec; A wave, 0.12 ± 1.96 cm/sec and 0.64 ± 4.54 cm/sec.

The variation of echocardiographic characteristics from rest to exercise is reported in Table 2. HCM patients performed a maximum workload of 100 W (IQR 75-125) with a median heart rate increase from 73 (IQR 66-84) bpm to 128 (IQR 112-142) bpm. Median LV outflow



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3 gradient increased from 11 (IQR 7-31) mmHg to 27 (IQR 16-98) mmHg on exercise; 15 patients
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5 (20%) without obstruction at rest developed a gradient ≥ 30 mmHg on exercise and 18 (24%) had
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7 an increase in outflow gradient ≥ 50 mmHg. In 28 patients (37%) EF did not increase or
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9 decreased with exercise.
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12 There was no correlation between the extent of fibrosis and maximum LVOT gradient during
13
14 exercise ($r = -0,197$, $p = 0,087$). Considering the variation in LVOT gradient during exercise, there
15
16 was a weak correlation with the extent of fibrosis in the overall population ($r = -0,243$, $p = 0,034$)
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18 and a stronger correlation in patients with an obstructive form of the disease at rest ($r = -0,524$,
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20 $p = 0,021$), (Figure 1).
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24 Patients with a maximum gradient during exercise ≥ 50 mmHg tended to have a lesser amount of
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26 fibrosis than those with a maximum gradient < 50 mmHg, the difference however did not reach
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28 significance (1,1% (IQR 0-3,9) vs 4,1% (IQR 0,5-8,2), $p = 0,089$). Patients with an increase in
29
30 LVOT gradient ≥ 50 mmHg had a significantly lesser extent of fibrosis than those with an
31
32 increase < 50 mmHg (0,7% (IQR 0-2,4) vs 3,2% (IQR 0,2-7,4), $p = 0,006$) (Figure 2). There was
33
34 no difference in terms of fibrosis extent between patients with an increase in LVOT gradient \geq or
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36 $<$ than the median value (14 mmHg) (1,7% (IQR 0-4,6) vs 2,8% (IQR 0-7,0), $p = 0,330$). When
37
38 dividing the population in to quartiles according to LVOT gradient increase during exercise the
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40 extent of fibrosis was significantly different: in patients with an increase < 8 mmHg the median
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42 value of fibrosis was 3,4% (IQR 2,2-8,6), in patients with an increase between 8 and 13 mmHg
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44 was 1,1% (IQR 0,0-6,6), in patients with an increase between 14 and 46 mmHg was 4,4% (IQR
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46 0,7-11,6) and in patients with an increase ≥ 47 mmHg median value of fibrosis was 0,6% (IQR
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48 0,0-2,4) ($p = 0,009$).
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DISCUSSION

This study shows that myocardial fibrosis (detected as LGE on MRI) may influence the development of LVOT gradient during exercise in patients with HCM and normal EF: patients with higher exercise-induced gradients show a lesser degree of myocardial fibrosis and vice versa (Figure 3). This negative association is more evident in patients with an obstructive form at rest.

In recent years, myocardial fibrosis has been emerging as an important actor in the complex pathophysiology of HCM. It has been suggested that impairment in collagen turnover could be a component of the disease phenotype and that it appears as an early manifestation of sarcomere gene mutations, before the development of overt LV hypertrophy [19-20]. When hypertrophy develops, increasing amounts of interstitial fibrosis can be detected noninvasively by gadolinium-enhanced cardiac MRI [6-8]. The exact mechanism leading to fibrosis remains unknown but it has been hypothesized that the main triggers for the fibrotic process include molecular factors at cellular level (induced by sarcomeric mutations), hemodynamic factors (overall ventricular afterload resulting from the sum of LV outflow obstruction and systolic blood pressure), and ischemia (mainly related to small intramural coronary vessel disease) [8]. Myocardial fibrosis in HCM has been associated with the risk of life-threatening arrhythmias and with a wide spectrum of systolic dysfunction, ranging from a mild LV EF reduction to the end stage phase [9-14]. The present study confirmed the association between myocardial fibrosis and contractility, assuming that LV systolic function is one of the major determinants of the LVOT gradient increase during effort. The prevalence of myocardial fibrosis (71%) in our study is comparable with that of the largest published series [12, 14], in most cases however, LGE was modest and presented a patchy distribution. Our results therefore support the concept of a



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3 continuum of hemodynamic effect of myocardial fibrosis on LV function. Large "scar-like" areas
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5 of fibrosis are a determinant of the end-stage evolution, lesser degrees of fibrosis are associated
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7 with slight EF reduction [14, 15], while even lesser degrees of fibrosis, while not influencing EF
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9 at rest, seem to result in a lesser contractility recruitment during exercise, leading to a lower LV
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11 outflow gradient. Notably, the effects of myocardial fibrosis were particularly evident among
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13 patients with LV outflow gradient already present at rest. Indeed LV contractility is not the only
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15 determinant of LV outflow obstruction; excessive length of the anterior mitral leaflet,
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17 abnormalities in the subvalvular apparatus and load conditions also play a role [21]. In patients
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19 with no LV outflow obstruction at rest (related for example to the large anatomical size of LVOT
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21 and/or a non-redundant mitral valve), the increase in contractility could fail to generate a
22
23 significant LV gradient increase regardless of the amount of myocardial fibrosis.
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32 **Study limitations**

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34 When interpreting our findings one must consider the low absolute number of patients as well as
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36 the fact that the results derive from the analysis of multiple subgroups, even though these were
37
38 identified with a solid pathophysiological rationale.
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41 The lack of direct hemodynamic measurement of LV pressures limits the pathophysiological
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43 interpretation of our data which is essentially based on the behavior of LV outflow gradient and
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45 indexes of ventricular and myocardial function. Also, our study did not include a detailed
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47 analysis of the behaviour of LV volumes during exercise and of their correlation with other
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49 variables. Indeed, the small absolute values of LV end-systolic volume in this disease during
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51 exercise (often below the repeatability threshold) make echocardiography an unreliable
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53 technique for this purpose.
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CONCLUSIONS

In patients with HCM and normal EF at rest, myocardial fibrosis —detected by MRI— is associated with a lower increase in LVOT gradient during exercise, probably due to a lesser degree of myocardial contractility recruitment. This negative association is more evident in patients with an obstructive form at rest.

COMPETING INTERESTS

The authors have no competing interests to declare.

CONTRIBUTIONS

GR, LL, FL, SR, CP, FP, MLBR, RF: substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; EB, ML, IO: drafting the article or critical revision; and CR: final approval of the manuscript

FUNDING.

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

DATA SHARING STATEMENT

No additional data.



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Table 1. Baseline clinical, echocardiographic and magnetic resonance characteristics.

<i>Clinical</i>	
No. of patients, n (%)	76
Males, n (%)	51 (67%)
Age, years	48 (41-61)
Family history of HC, n (%)	34 (45%)
Family history of SD, n (%)	10 (13%)
NYHA functional class I, n (%)	61 (80%)
II, n (%)	14 (18%)
Unexplained syncope, n (%)	12 (16%)
NSVT on Holter monitor, n (%)	21 (28%)
<i>Echocardiography:</i>	
LV gradient ≥ 30 mmHg at rest, n (%)	20 (26%)
Maximum WT, mm	20 (17-23)
Maximum WT ≥ 30 mm, n (%)	3 (4%)
Left atrium diameter, mm	43 (39-48)
<i>Magnetic resonance imaging:</i>	
LV mass, g/m ²	155 (124-196)
LV mass/end-diastolic volume, g/ml	1.09 (0.92-1.46)
LGE % of LV mass, (%)	2.4 (0-6)



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3 Legend: HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LV left
4 ventricle; NSVT, non-sustained ventricular tachycardia; NYHA: New York Heart Association;
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8 SD: sudden death; WT: wall thickness.
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10 Continuous variables are expressed as median values (interquartile range, IQR).
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Table 2. Echocardiographic data at rest and during exercise.

	Rest	Exercise	p value
Maximum workload, W		100 (75, 125)	
Heart rate, bpm	73 (66, 84)	128 (112, 142)	<0.001
Left ventricle outflow gradient, mmHg	11 (7, 31)	27 (16, 98)	<0.001
Δ Left ventricle outflow gradient, mmHg		14 (8, 46)	
Mitral regurgitation jet area, cm ²	1.2 (0.1, 3.1)	3.0 (0.6, 7.1)	<0.001
Δ Mitral regurgitation jet area, cm ²		0.6 (0, 3.6)	
End-diastolic volume, ml/m ²	35 (28, 45)	29 (20, 40)	<0.001
Δ End-diastolic volume, ml/m ²		-6 (-12, -2)	
End-systolic volume, ml/m ²	8 (5, 12)	5 (3, 7)	<0.001
Δ End-systolic volume, ml/m ²		-3 (-5, 0)	
Stroke volume, ml/m ²	28 (22, 37)	23 (17, 32)	<0.001
Δ Stroke volume, ml/m ²		-4 (-9, 1)	
Ejection fraction, %	78 (71, 84)	83 (75, 88)	<0.001
Δ Ejection fraction, %		5 (-2, 11)	
E wave, cm/s	71 (59, 89)	97 (83, 121)	<0.001
Δ E wave, cm/s		25 (8, 44)	
A wave, cm/s	73 (60, 91)	104 (84, 125)	<0.001
Δ A wave, cm/s		27 (8, 45)	
Deceleration time, ms	185 (160, 250)	N.A.	/
S wave, cm/s	7.5 (6.1, 9.0)	9.4 (7.6, 11.9)	<0.001
Δ S wave, cm/s		2.1 (0.7, 3.3)	
E' wave, cm/s	7.5 (5.9, 9.0)	9.7 (7.4, 13.8)	<0.001
Δ E' wave, cm/s		3.1 (0.9, 5.0)	
A' wave, cm/s	8.4 (6.5, 11.1)	11.1 (9.3, 15.5)	<0.001
Δ A' wave, cm/s		2.5 (0.9, 5.1)	
E/E'	9.9 (7.0, 14.2)	9.2 (7.1, 12.8)	0.270



Figure legends

Figure 1. Linear regression analysis between extent of fibrosis and changes in LV outflow tract gradient during exercise. A: in the overall population, B: in patients with obstructive HCM at rest.

Note: the mark indicated by the arrow represents four patients that showed no LGE and an increase in LVOT gradient during exercise of 50, 50, 51 and 52 mmHg respectively.

Figure 2. Fibrosis extent (expressed as median and interquartile range) in patients with an increase in exercise gradient $<$ or \geq 50 mmHg.

Figure 3. Myocardial fibrosis and changes in LV outflow tract gradient during exercise. A: patient with a large amount of myocardial fibrosis and modest increase in LV outflow tract gradient. B: patient with a limited amount of fibrosis and relevant increase in LV outflow tract gradient during exercise.



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3 **Effects of myocardial fibrosis assessed by magnetic resonance imaging on dynamic left**
4 **ventricular outflow tract obstruction in patients with hypertrophic cardiomyopathy: a**
5 **retrospective database analysis.**
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BACKGROUND

The pathophysiology of hypertrophic cardiomyopathy (HCM) is the result of a number of interrelated factors that include impaired ventricular relaxation, increased myocardial stiffness, myocardial ischemia, left ventricular outflow tract (LVOT) obstruction and mitral regurgitation [1, 2]. In recent years magnetic resonance imaging (MRI) and exercise echocardiography have opened new possibilities for non-invasive evaluation of myocardial substrate and LV function in HCM [3-8]. In particular, gadolinium-enhanced MRI has shown that a high percentage of patients with HCM has variable degrees of late gadolinium enhancement (LGE) which, in this disease, has been shown to correspond to interstitial fibrosis [6-9]. Myocardial fibrosis in HCM has been shown to be associated with an increased incidence of sudden death risk factors—particularly ventricular arrhythmias— [9-11] and an increased risk of HCM-related morbidity and mortality [12-14]. Additionally, myocardial fibrosis influences LV function at rest negatively [15-16]. Not only are large confluent areas of LGE associated with the end-stage phase of the disease, but also lesser amounts of fibrosis seem to determine a reduction in LV systolic function at rest (albeit within the “normal” ejection fraction (EF) range) [15]. The relationship between myocardial fibrosis and LV function during exercise remains unexplored. Since LVOT gradient during exercise is mainly related to the increase in LV contractility, we designed a hypothesis generating study to explore the relationship between MRI assessed myocardial fibrosis and LV outflow gradient during exercise echocardiography in HCM patients with normal LV EF.

METHODS

Patients



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Ninety-one consecutive outpatients, evaluated at the S. Orsola-Malpighi University Hospital Bologna, Italy between January 2009 and November 2010, were considered for the study. Fifteen patients were excluded due to coexistent coronary artery disease (4 patients), atrial fibrillation (n=3), previous surgical septal myectomy (n=1), LV EF <50% at rest (n=2) and general contraindications / refusal to MRI (n=5). All patients underwent contrast-enhanced cardiac MRI and bicycle exercise echocardiogram within a 1 month period. All patients fulfilled conventional criteria for HCM with LV hypertrophy ≥ 15 mm [17].

All patients provided written informed consent for exercise echocardiography and magnetic resonance. No specific ethical approval was required for this study that included only non-invasive examinations that HCM patients routinely undergo at our institution.

Exercise echocardiography protocol

Having suspended beta-blockers and/or calcium antagonist and dysopyramide for at least 5 half-lives, patients performed a symptom-limited bicycle exercise stress test in semi supine position on an exercise echo-tilting table (stress echo supine ergometer, Ergoselect 1200 EL, Ergoline GmbH, Bitz, Germany). The workload was increased by 25 watts every 2 minutes. Blood pressure and 12-lead ECG were recorded every minute. Echocardiographic images were assessed at baseline and at peak exercise using a Philips Sonos 5500 Ultrasound System (Philips Ultrasound, Andover, MA, USA) equipped with a harmonic fusion imaging probe (s3) and off-line cineloop analysis software. All images were recorded digitally and analysed off-line and each parameter was measured on an average of three consecutive beats both at rest and during exercise. LV volumes and EF were calculated using the Simpson method from the apical 4 and 2-chamber view. LV volumes were normalized to the body surface area. Mitral regurgitation was



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3 quantified with the colour-area method. Continuous wave Doppler was used to measure LV
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5 outflow gradient from the apical 4-chamber view. The early filling (E) and late (A) filling
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7 velocities, as well as the deceleration time (DT) of early filling, were measured from the
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9 transmitral flow. Tissue Doppler velocities were recorded from the medial (septal) and lateral
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11 mitral annulus as previously reported [18] and averaged; the ratio of early mitral diastolic inflow
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13 velocity to early diastolic mitral annular velocity (E/E') was calculated.
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20 **MRI technique**

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22 MRI was performed on a 1.5 T scanner (Signa Twin Speed Excite, General Electric, Milwaukee,
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24 WI, USA) with surface coils and prospective ECG triggering. LV end-systolic and end-diastolic
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26 diameters as well as maximal (end-diastolic) wall thickness were traced and recorded from the
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28 short axis and long axis views (8 mm slice thickness, no gap) of the standard ECG-gated steady
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30 state free precession (SSFP) cine sequence. Image parameters were: repetition time of 3.5 msec,
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32 echo time 1.6 msec, temporal resolution 40 msec, matrix 224 x 160, flip angle 45°, bandwidth
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34 125 kHz, views per segment 8 to 16. LV volumes, mass and EF were measured from a stack of
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36 sequential 8 mm short axis slices (no gap) from the atrio-ventricular ring to the apex, through
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38 analysis with a commercially available software (Mass Analysis Plus, Medis, Leiden, The
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40 Netherlands) and were indexed to body surface in m². LGE images for detection of delayed
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42 hyper-enhancement were acquired 10-15 minutes after intravenous administration of
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44 Gadopentate dimeglumine (0.2 mmol/kg) (Magnevist; Schering, Berlin, Germany) using a
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46 breath-hold segmented inversion recovery fast gradient echo sequence in the short axis and in
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48 long axis planes of the LV, with 9 mm slice thickness and no gap. Image parameters were:
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50 repetition time of 5.3 ms, echo time 1.3 ms, flip angle 20°, matrix 256 x 160, NEX 2 and field of
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3 view 320 mm. Optimal inversion time to null normal myocardial signal was determined for each
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5 patient and ranged from 220 to 320 ms. After visual inspection of all short axis LV slices to
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7 identify areas of completely nulled myocardium (normal myocardium), the mean signal intensity
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9 of normal myocardial tissue was calculated and a threshold ≥ 2 standard deviations exceeding the
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11 mean was used to identify LGE areas. This limit was deemed acceptable to discriminate LGE
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13 from healthy myocardium without reducing sensibility. LGE areas were outlined manually and
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15 the total volume (expressed in grams) was quantified using a specific software (ReportCard, GE
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17 Medical Systems, Milwaukee, WI, USA) and expressed as percentage of LV mass. LGE analysis
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19 was performed by one experienced reader (L.L., > 8 years of MRI experience) and reviewed by a
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21 second reader (R.F., > 10 years of MRI experience).
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29 **Study design and statistical analysis**

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31 In order to explore a possible relation between myocardial fibrosis and LV outflow obstruction
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33 during exercise the following analyses were planned:
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36 • Linear regression analysis between the extent of fibrosis and maximum LVOT gradient
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38 during exercise;
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40 • Linear regression analysis between the extent of fibrosis and changes in LVOT gradient
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42 during exercise (in the overall population and in the patients with an obstructive form at rest,
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44 defined as LV gradient ≥ 30 mmHg);
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46 • Comparison of fibrosis extent between patients with maximum gradient \geq or $<$ 50 mmHg,
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48 and between patients with an increase in exercise gradient \geq or $<$ 50 mmHg,
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52 • Comparison of fibrosis extent between patients with a gradient increase above or below the
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54 median value in our population, and among different quartiles of gradient increase.
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Categoric variables are expressed as total numbers and percentages. Continuous variables are expressed as median values (interquartile range, IQR). Comparison of categoric variables was performed with the chi-square test and continuous variables were analyzed with the Mann-Whitney U test, Wilcoxon signed-rank test and Kruskal-Wallis test as appropriate. A p value of 0.05 was considered to be statistically significant. Regarding echocardiographic measurements, intra-observer variability was assessed in two different blind evaluations 30 days apart, whereas inter-observer variability was assessed by two different observers (G.R. and E.B.). Both assessments were made on a 15 patient sample. Data processing and statistical analyses were performed using the SPSS 15.0 statistical program (SPSS Inc., Chicago, IL, USA).

RESULTS

LGE was present in 54 patients (71%), involving a percentage of LV mass ranging from 0,2% to 32,4%. Fibrosis consisted of small, diffuse areas in 32 patients (59%) and was confluent into a smaller number of larger areas in 22 patients (41%). Table 1 reports the clinical, resting echocardiographic and MRI characteristics of the study population. Regarding echocardiographic measurements, mean intra-observer variability for end-diastolic and for end-systolic volume were 4 ± 1 ml/m² and 3 ± 1 ml/m² respectively. Mean inter-observer variability for end-diastolic and for end-systolic volume were 5 ± 1 ml/m² and 4 ± 1 ml/m² respectively. Mean intra-observer and inter-observer variability of Doppler indexes of LV filling were as follows: E wave, 0.08 ± 2.36 cm/sec and 1.20 ± 4.30 cm/sec; A wave, 0.12 ± 1.96 cm/sec and 0.64 ± 4.54 cm/sec.

The variation of echocardiographic characteristics from rest to exercise is reported in Table 2. HCM patients performed a maximum workload of 100 W (IQR 75-125) with a median heart rate increase from 73 (IQR 66-84) bpm to 128 (IQR 112-142) bpm. Median LV outflow



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gradient increased from 11 (IQR 7-31) mmHg to 27 (IQR 16-98) mmHg on exercise; 15 patients (20%) without obstruction at rest developed a gradient ≥ 30 mmHg on exercise and 18 (24%) had an increase in outflow gradient ≥ 50 mmHg. In 28 patients (37%) EF did not increase or decreased with exercise.

There was no correlation between the extent of fibrosis and maximum LVOT gradient during exercise ($r = -0,197$, $p = 0,087$). Considering the variation in LVOT gradient during exercise, there was a weak correlation with the extent of fibrosis in the overall population ($r = -0,243$, $p = 0,034$) and a stronger correlation in patients with an obstructive form of the disease at rest ($r = -0,524$, $p = 0,021$), (Figure 1).

Patients with a maximum gradient during exercise ≥ 50 mmHg tended to have a lesser amount of fibrosis than those with a maximum gradient < 50 mmHg, the difference however did not reach significance (1,1% (IQR 0-3,9) vs 4,1% (IQR 0,5-8,2), $p = 0,089$). Patients with an increase in LVOT gradient ≥ 50 mmHg had a significantly lesser extent of fibrosis than those with an increase < 50 mmHg (0,7% (IQR 0-2,4) vs 3,2% (IQR 0,2-7,4), $p = 0,006$) (Figure 2). There was no difference in terms of fibrosis extent between patients with an increase in LVOT gradient \geq or $<$ than the median value (14 mmHg) (1,7% (IQR 0-4,6) vs 2,8% (IQR 0-7,0), $p = 0,330$). When dividing the population in to quartiles according to LVOT gradient increase during exercise the extent of fibrosis was significantly different: in patients with an increase < 8 mmHg the median value of fibrosis was 3,4% (IQR 2,2-8,6), in patients with an increase between 8 and 13 mmHg was 1,1% (IQR 0,0-6,6), in patients with an increase between 14 and 46 mmHg was 4,4% (IQR 0,7-11,6) and in patients with an increase ≥ 47 mmHg median value of fibrosis was 0,6% (IQR 0,0-2,4) ($p = 0,009$).



DISCUSSION

This study shows that myocardial fibrosis (detected as LGE on MRI) may influence the development of LVOT gradient during exercise in patients with HCM and normal EF: patients with higher exercise-induced gradients show a lesser degree of myocardial fibrosis and vice versa (Figure 3). This negative association is more evident in patients with an obstructive form at rest.

In recent years, myocardial fibrosis has been emerging as an important actor in the complex pathophysiology of HCM. It has been suggested that impairment in collagen turnover could be a component of the disease phenotype and that it appears as an early manifestation of sarcomere gene mutations, before the development of overt LV hypertrophy [19-20]. When hypertrophy develops, increasing amounts of interstitial fibrosis can be detected noninvasively by gadolinium-enhanced cardiac MRI [6-8]. The exact mechanism leading to fibrosis remains unknown but it has been hypothesized that the main triggers for the fibrotic process include molecular factors at cellular level (induced by sarcomeric mutations), hemodynamic factors (overall ventricular afterload resulting from the sum of LV outflow obstruction and systolic blood pressure), and ischemia (mainly related to small intramural coronary vessel disease) [8]. Myocardial fibrosis in HCM has been associated with the risk of life-threatening arrhythmias and with a wide spectrum of systolic dysfunction, ranging from a mild LV EF reduction to the end stage phase [9-14]. The present study confirmed the association between myocardial fibrosis and contractility, assuming that LV systolic function is one of the major determinants of the LVOT gradient increase during effort. The prevalence of myocardial fibrosis (71%) in our study is comparable with that of the largest published series [12, 14], in most cases however, LGE was modest and presented a patchy distribution. Our results therefore support the concept of a



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3 continuum of hemodynamic effect of myocardial fibrosis on LV function. Large "scar-like" areas
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5 of fibrosis are a determinant of the end-stage evolution, lesser degrees of fibrosis are associated
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7 with slight EF reduction [14, 15], while even lesser degrees of fibrosis, while not influencing EF
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9 at rest, seem to result in a lesser contractility recruitment during exercise, leading to a lower LV
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11 outflow gradient. Notably, the effects of myocardial fibrosis were particularly evident among
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13 patients with LV outflow gradient already present at rest. Indeed LV contractility is not the only
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15 determinant of LV outflow obstruction; excessive length of the anterior mitral leaflet,
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17 abnormalities in the subvalvular apparatus and load conditions also play a role [21]. In patients
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19 with no LV outflow obstruction at rest (related for example to the large anatomical size of LVOT
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21 and/or a non-redundant mitral valve), the increase in contractility could fail to generate a
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23 significant LV gradient increase regardless of the amount of myocardial fibrosis.
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32 **Study limitations**

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34 **When interpreting our findings one must consider the low absolute number of patients as well as**
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36 **the fact that the results derive from the analysis of multiple subgroups, even though these were**
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38 **identified with a solid pathophysiological rationale.**
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41 The lack of direct hemodynamic measurement of LV pressures limits the pathophysiological
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43 interpretation of our data which is essentially based on the behavior of LV outflow gradient and
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45 indexes of ventricular and myocardial function. Also, our study did not include a detailed
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47 analysis of the behaviour of LV volumes during exercise and of their correlation with other
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49 variables. Indeed, the small absolute values of LV end-systolic volume in this disease during
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51 exercise (often below the repeatability threshold) make echocardiography an unreliable
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53 technique for this purpose.
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CONCLUSIONS

In patients with HCM and normal EF at rest, myocardial fibrosis —detected by MRI— is associated with a lower increase in LVOT gradient during exercise, probably due to a lesser degree of myocardial contractility recruitment. This negative association is more evident in patients with an obstructive form at rest.

COMPETING INTERESTS

The authors have no competing interests to declare.

CONTRIBUTIONS

GR, LL, FL, SR, CP, FP, MLBR, RF: substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; EB, ML, IO: drafting the article or critical revision; and CR: final approval of the manuscript

FUNDING.

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Table 1. Baseline clinical, echocardiographic and magnetic resonance characteristics.

<i>Clinical</i>	
No. of patients, n (%)	76
Males, n (%)	51 (67%)
Age, years	48 (41-61)
Family history of HC, n (%)	34 (45%)
Family history of SD, n (%)	10 (13%)
NYHA functional class I, n (%)	61 (80%)
II, n (%)	14 (18%)
Unexplained syncope, n (%)	12 (16%)
NSVT on Holter monitor, n (%)	21 (28%)
<i>Echocardiography:</i>	
LV gradient ≥ 30 mmHg at rest, n (%)	20 (26%)
Maximum WT, mm	20 (17-23)
Maximum WT ≥ 30 mm, n (%)	3 (4%)
Left atrium diameter, mm	43 (39-48)
<i>Magnetic resonance imaging:</i>	
LV mass, g/m ²	155 (124-196)
LV mass/end-diastolic volume, g/ml	1.09 (0.92-1.46)
LGE % of LV mass, (%)	2.4 (0-6)



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3 Legend: HCM, hypertrophic cardiomyopathy; LGE, late gadolinium enhancement; LV left
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5 ventricle; NSVT, non-sustained ventricular tachycardia; NYHA: New York Heart Association;
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8 SD: sudden death; WT: wall thickness.
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10 Continuous variables are expressed as median values (interquartile range, IQR).
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Table 2. Echocardiographic data at rest and during exercise.

	Rest	Exercise	p value
Maximum workload, W		100 (75, 125)	
Heart rate, bpm	73 (66, 84)	128 (112, 142)	<0.001
Left ventricle outflow gradient, mmHg	11 (7, 31)	27 (16, 98)	<0.001
Δ Left ventricle outflow gradient, mmHg		14 (8, 46)	
Mitral regurgitation jet area, cm ²	1.2 (0.1, 3.1)	3.0 (0.6, 7.1)	<0.001
Δ Mitral regurgitation jet area, cm ²		0.6 (0, 3.6)	
End-diastolic volume, ml/m ²	35 (28, 45)	29 (20, 40)	<0.001
Δ End-diastolic volume, ml/m ²		-6 (-12, -2)	
End-systolic volume, ml/m ²	8 (5, 12)	5 (3, 7)	<0.001
Δ End-systolic volume, ml/m ²		-3 (-5, 0)	
Stroke volume, ml/m ²	28 (22, 37)	23 (17, 32)	<0.001
Δ Stroke volume, ml/m ²		-4 (-9, 1)	
Ejection fraction, %	78 (71, 84)	83 (75, 88)	<0.001
Δ Ejection fraction, %		5 (-2, 11)	
E wave, cm/s	71 (59, 89)	97 (83, 121)	<0.001
Δ E wave, cm/s		25 (8, 44)	
A wave, cm/s	73 (60, 91)	104 (84, 125)	<0.001
Δ A wave, cm/s		27 (8, 45)	
Deceleration time, ms	185 (160, 250)	N.A.	/
S wave, cm/s	7.5 (6.1, 9.0)	9.4 (7.6, 11.9)	<0.001
Δ S wave, cm/s		2.1 (0.7, 3.3)	
E' wave, cm/s	7.5 (5.9, 9.0)	9.7 (7.4, 13.8)	<0.001
Δ E' wave, cm/s		3.1 (0.9, 5.0)	
A' wave, cm/s	8.4 (6.5, 11.1)	11.1 (9.3, 15.5)	<0.001
Δ A' wave, cm/s		2.5 (0.9, 5.1)	
E/E'	9.9 (7.0, 14.2)	9.2 (7.1, 12.8)	0.270

Figure legends

Figure 1. Linear regression analysis between extent of fibrosis and changes in LV outflow tract gradient during exercise. A: in the overall population, B: in patients with obstructive HCM at rest.

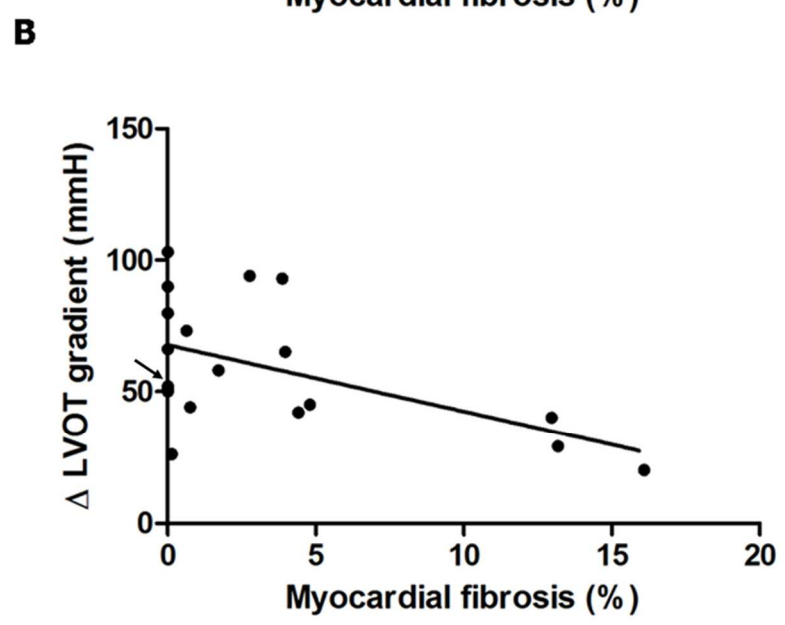
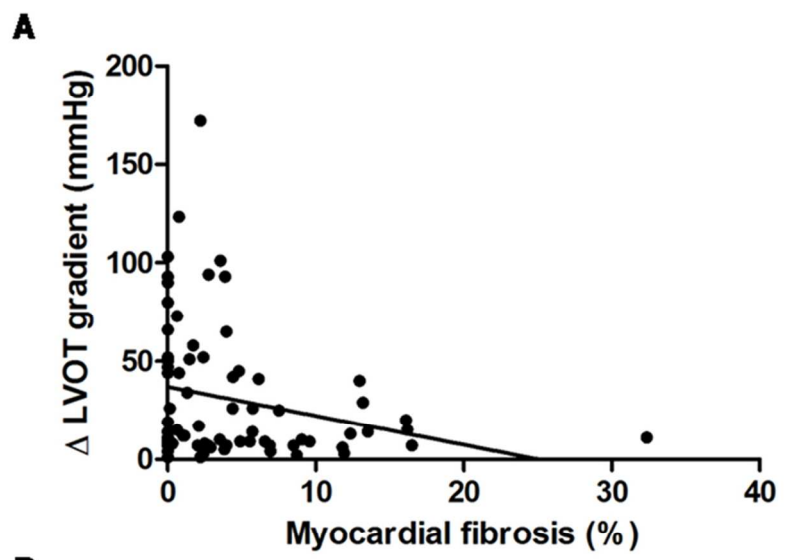
Note: the mark indicated by the arrow represents four patients that showed no LGE and an increase in LVOT gradient during exercise of 50, 50, 51 and 52 mmHg respectively.

Figure 2. Fibrosis extent (expressed as median and interquartile range) in patients with an increase in exercise gradient $<$ or \geq 50 mmHg.

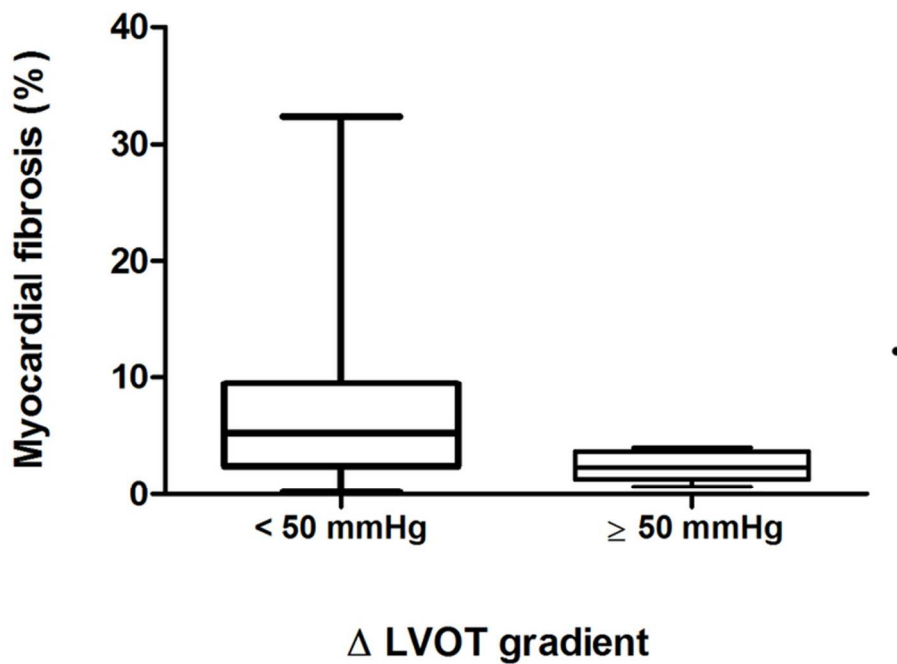
Figure 3. Myocardial fibrosis and changes in LV outflow tract gradient during exercise. A: patient with a large amount of myocardial fibrosis and modest increase in LV outflow tract gradient. B: patient with a limited amount of fibrosis and relevant increase in LV outflow tract gradient during exercise.



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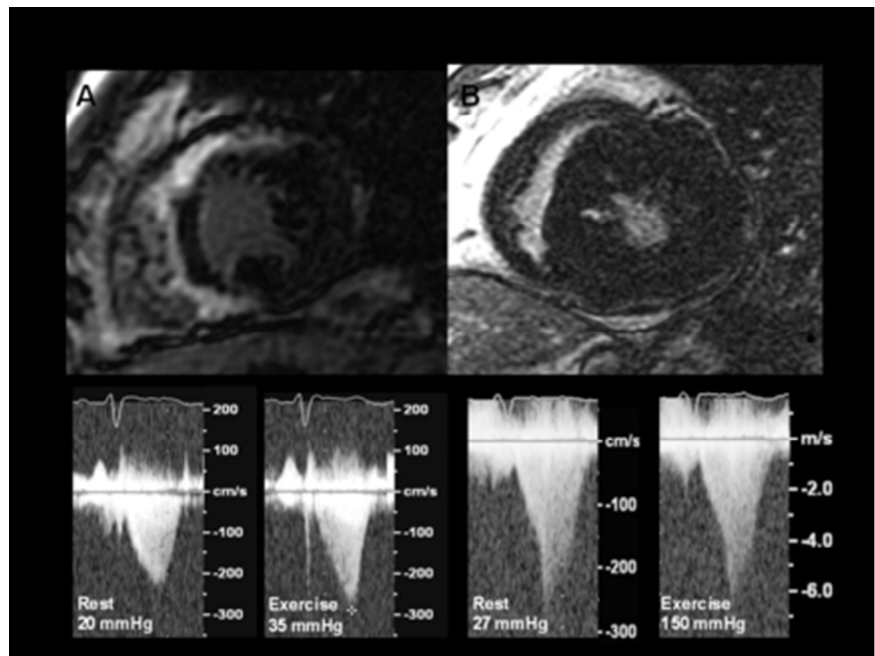


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STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers of outcome events or summary measures
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.