






Paradoxical prolongation of QT interval during exercise in patients with hypertrophic cardiomyopathy: cellular mechanisms and implications for diastolic function

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Aims

Ventricular cardiomyocytes from hypertrophic cardiomyopathy (HCM) patient hearts show prolonged action potential duration (APD), impaired intracellular Ca^{2+} homeostasis and abnormal electrical response to beta-adrenergic stimulation. We sought to determine whether this behaviour is associated with abnormal changes of repolarization during exercise and worsening of diastolic function, ultimately explaining the intolerance to exercise experienced by some patients without obstruction.

Methods and results

Non-obstructive HCM patients (178) and control subjects (81) underwent standard exercise testing, including exercise echocardiography. Ventricular myocytes were isolated from myocardial samples of 23 HCM and eight non-failing non-hypertrophic surgical patients. The APD shortening in response to high frequencies was maintained in HCM myocytes, while β -adrenergic stimulation unexpectedly prolonged APDs, ultimately leading to a lesser shortening of APDs in response to exercise. In HCM vs. control subjects, we observed a lesser shortening of QT interval at peak exercise (QTc: $+27 \pm 52$ ms in HCM, -4 ± 50 ms in controls, $P < 0.0001$). In patients showing a marked QTc prolongation (>30 ms), the excessive shortening of the electrical diastolic period was linked with a limited increase of heart-rate and deterioration of diastolic function at peak effort.

Conclusions

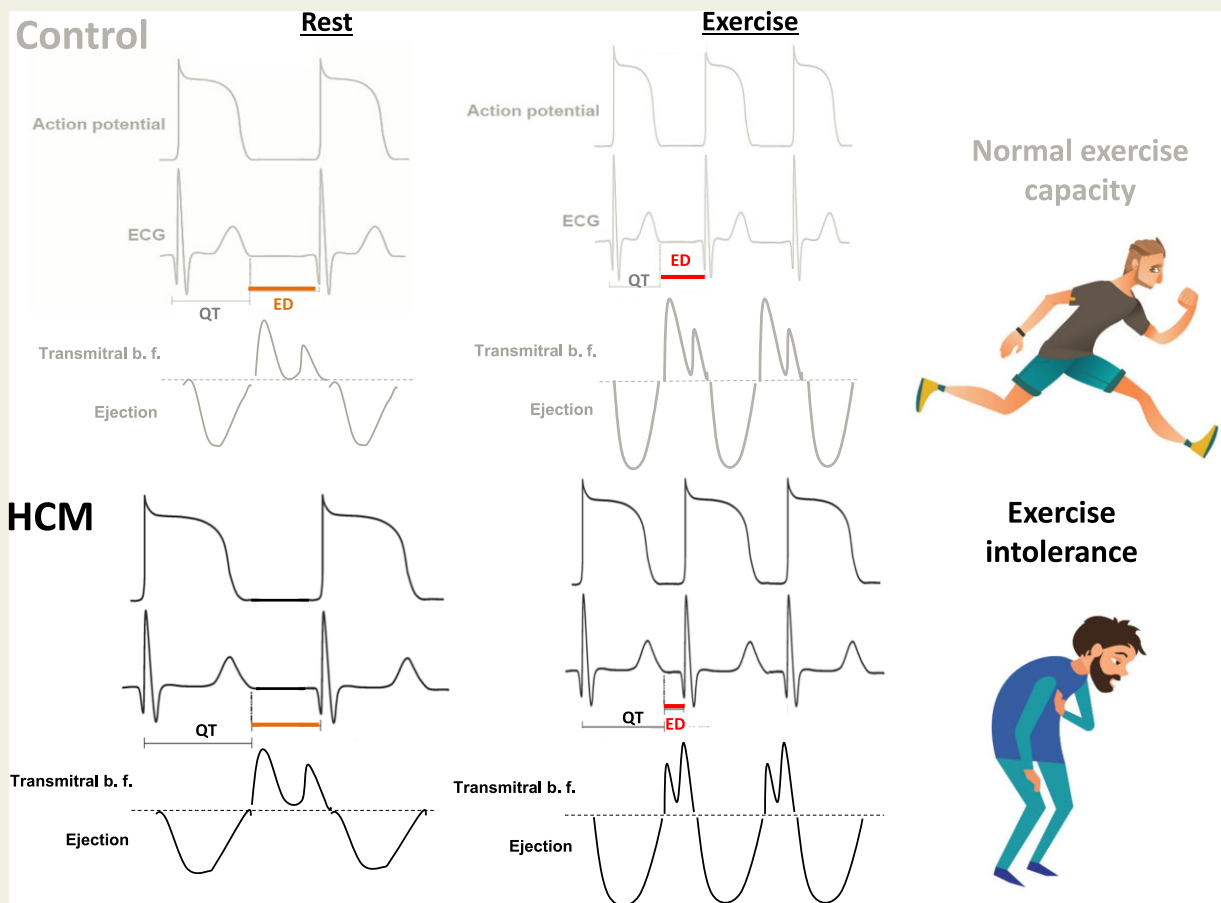
Abnormal balance of Ca^{2+} - and K^{+} -currents in HCM cardiomyocytes determines insufficient APD and Ca^{2+} -transient shortening with exercise. In HCM patients, exercise-induced QTc prolongation was associated with impaired diastolic reserve, contributing to the reduced exercise tolerance. Our results support the idea that severe electrical cardiomyocyte abnormalities underlie exercise intolerance in a subgroup of HCM patients without obstruction.

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Graphical Abstract



Action potentials, QT/ED intervals, and relaxation mechanics. Scheme depicting the relationship between action potential duration at rest and at peak exercise, the changes in the duration of QT and electrical diastolic (ED) intervals during exercise and their possible impact on cardiac mechanics. Notably, the insufficient shortening of APs at peak exercise leads to a lower degree of QT shortening in HCM patients, causing excessive shrinking of the electrical diastole.

Keywords

Cardiomyocytes • Action potential • Beta adrenergic receptor • Cardiac relaxation • Repolarization • Stress echocardiography

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease of the myocardium with heterogeneous presentation and outcome.¹ Reduced exercise tolerance, due to the occurrence of congestive symptoms, dyspnoea or chest pain during exercise, is a common symptom in HCM patients, with marked impact on their quality of life. While the mechanisms leading to exertional symptoms in patients with obstruction of the left ventricle outflow tract (LVOT) are well-established, the cause of exercise-induced symptoms in non-obstructive patients is much less defined. It is generally attributed to the worsening of diastolic function during exercise, but the mechanisms leading to exercise-induced diastolic dysfunction remain undefined. Indeed, many gaps remain in our understanding of cellular mechanisms underlying key pathophysiological features in the disease, such as arrhythmic propensity and diastolic dysfunction, and the response of HCM myocardium to neurohormonal stimuli or fast rates has never been studied in detail. We previously analyzed

the electromechanical profile of cardiomyocytes isolated from myectomy samples of patients with obstructive HCM.² HCM cardiomyocytes showed prolonged action potentials (APs), down-regulation of K^+ currents, a marked overexpression of the depolarizing late Na^+ current (I_{NaL}), and a significant reduction of K^+ repolarizing currents,² often associated with delayed relaxation due to slower Ca^{2+} -transients and elevated diastolic Ca^{2+} . Furthermore, we recently observed that β -adrenergic stimulation with isoproterenol (ISO) at a fixed stimulation rate causes a paradoxical prolongation of APs in human HCM ventricular cardiomyocytes, while it slightly shortens APs in non-failing non-hypertrophic human cells.³ Beta-adrenergic stimulation is expected to increase the amplitude and shorten the duration of intracellular Ca^{2+} transients in cardiac myocytes. However, the abnormal electrical behaviour observed in HCM myocytes is associated with an insufficient shortening of Ca^{2+} transients duration in the presence of ISO.³ The net

expected effect of these changes on left ventricular (LV) mechanics would be a delay of LV relaxation during exercise, causing a loss of diastolic reserve, with increasing degrees of diastolic dysfunction during exercise or stress, especially at faster heart rates (HRs). A detailed *in vitro* analysis of the response of human HCM myocardium to exercise-like conditions has never been performed before.

Exercise echocardiography is commonly performed as a routine test in patients with HCM, primarily to measure dynamic LVOT gradients provoked by physiological exercise, and for functional evaluation. In addition, exercise echocardiography provides important information regarding diastolic reserve.⁴ At the same time, electrocardiographic recordings during exercise allow the evaluation of QTc variability,⁵ as well as measuring changes of the electrical diastolic (ED) interval, that is, the time between the end of the T wave and the beginning of the next QRS complex. A detailed study assessing the changes in diastolic function and electrocardiogram (ECG) features during exercise tests has never been accomplished before in HCM patients.

In the present study, we therefore sought to determine the effects of simulated exercise *in vitro*, obtained with a combination of ISO and high-frequency stimulation, using ventricular myocytes and trabeculae isolated from ventricular samples of patients with HCM undergoing surgical myectomy, compared with septal samples from

non-hypertrophic non-failing surgical samples with mitral valve disease. In particular, we sought to determine the effects of simulated exercise on AP duration (APD), Ca²⁺ transients amplitude and kinetics and isometric twitch force. To validate the molecular determinants of the observed abnormalities, we performed ion current recordings and detailed measurements of intracellular [Ca²⁺] changes, and fitted the results into a validated mathematical model of HCM myocytes.^{6,7} In parallel, we conducted a clinical study in non-obstructive HCM patients undergoing exercise ECG and echocardiography tests, aimed at correlating exercise-induced changes of QTc interval with abnormalities of diastolic function.

Methods

Detailed methods description is in the [Supplementary material online, Supplemental Methods](#) patient selection.

In vitro studies

In vitro studies were performed at the University of Florence. Protocols were approved by the ethical committee of Careggi University-Hospital (2006/0024713; renewed May 2009). We enrolled 23 HCM patients regularly followed at our Cardiomyopathy Unit and consecutively referred to surgical myectomy for relief of drug-refractory symptoms related to LVOT obstruction, from January 2012 to October 2019. Among the 23 patients, 15 agreed to undergo mutational screening in sarcomeric genes. Clinical and genetic data are found in [Supplementary material online, Table S1](#). The control cohort comprised eight patients aged <75 years undergoing heart surgery for aortic stenosis or regurgitation and who required a septal myectomy operation due to the presence of a bulging septum causing symptomatic obstruction. All control patients had septal thickness <15 mm and preserved left-ventricular systolic function (ejection fraction >55%). Clinical data from non-failing non-hypertrophic valve-disease (NFNH-VD) patients are found in [Supplementary material online, Table S1](#). Notably, β -blocker treatment was withdrawn at least 48 h (>3 half-lives) before the intervention in all patients (HCM and NFNH-VD).

Clinical study

The clinical study was performed at the Careggi University Hospital of Florence, Italy. Protocols were approved by the ethical committee of Careggi University-Hospital (2006/0024713; renewed May 2009 and April 2016). We recruited 178 patients with a diagnosis of non-obstructive HCM⁸ and 81 control subjects without any cardiac diseases, consecutively undergoing exercise testing (2008–2019). We excluded all patients who could not achieve maximal or submaximal effort, that is, patients who did not meet these two criteria: (i) peak HR increased by more than 80% of the HR at rest, and (ii) peak HR was at least 67% of the maximal HR (220-age). Of note, 253 HCM patients undergoing exercise tests were initially screened and 178 met the abovementioned inclusion criteria (70%). We also excluded patients who developed maximal LVOT gradients equal or higher than 30 mmHg, either during the Valsalva manoeuvre or during the exercise echo test. Detailed clinical features from study patients can be found in [Table 1](#). Due to the lack of obstruction, none of these patients underwent surgical myectomy, therefore the patients recruited for the clinical study are different from those recruited for *in vitro* studies in cardiac samples.

Table 1 Clinical study patients' characteristics

	HCM (n = 178)	Control (n = 81)	P vs. CTRL
<i>Characteristics of clinical study population</i>			
Age	45 ± 15	45 ± 7	>0.05
Females	62 (35%)	29 (36%)	>0.05
Family history of HCM	69 (39%)	0 (0%)	N/A
Family history of sudden death	34 (19%)	0 (0%)	N/A
NYHA class I	114 (64%)	81 (100%)	N/A
NYHA class II	64 (36%)	0 (0%)	N/A
Angina	57 (32%)	0 (0%)	N/A
Syncope	30 (17%)	0%	N/A
NSVT	30 (17%)	0%	N/A
Beta-blockers	116 (65%)	0 (0%)	N/A
<i>Hemodynamic and exercise parameters</i>			
Exercise time, min	11 ± 4	12 ± 5	>0.05
Peak SBP, mmHg	165 ± 28	160 ± 33	>0.05
Peak heart rate, beats/min	126 ± 20	137 ± 23	<0.01
% of maximum predicted heart rate	77 ± 13	89 ± 9	<0.0001
Peak METs	6.5 ± 1.6	9.8 ± 1.8	<0.0001

HCM, hypertrophic cardiomyopathy; METs, metabolic equivalents; NYHA, New York Heart Association; NSVT, non-sustained ventricular tachycardia; SBP, systolic blood pressure.

Estimated oxygen uptake (VO₂) at peak exercise was calculated using the standard ACSM equation for leg cycle ergometer exercise, that is: VO₂ = [10.8 × work rate (W)]/body mass (kg). METs at peak exercise were calculated by dividing estimated VO₂ by 3.5.

P calculated with Student's t-test (unpaired groups).

In vitro study on human cardiomyocytes/trabeculae

Tissue processing and cell isolation was performed as described.⁹ Patch-clamp and calcium-fluorescence studies were performed in freshly-isolated ventricular cardiomyocytes, while isometric twitch force was recorded from intact trabeculae dissected from septal samples.^{2,3,7,10} ISO was used at 100 nM, to obtain a near-maximal activation of β -adrenergic receptors in cardiomyocytes, approximating the level of receptor activation during peak exercise activity.³ Data are expressed as mean \pm S.E.M. Statistical analysis was performed using multi-level statistics (linear mixed models) to encompass inter-patient variability, as previously described.^{2,3,7}

Mathematical modelling study

We used a population of calibrated mathematical models,^{6,11,12} incorporating a detailed model of the β -adrenergic stimulation response based on our *in vitro* results.¹³

Clinical study

178 HCM and 81 control subjects underwent standard bicycle ergometer exercise test. Routinely used medications were withdrawn at least three half-lives before the test, including beta blockers. At ECG, we recorded RR, QRS, Tpeak-Tend interval (Tp-e), JTp (J point to peak T wave), ED time (= RR interval-QT interval), QTc (QTc=QT/RR), and Δ QTc (QTc at peak exercise—rest QTc). Analysis of ECG traces and measurements of the intervals were performed by two independent blinded cardiologists with long-term experience in the interpretation of exercise ECG tests, and the two measured values were averaged to obtain each datapoint. In a subgroup of 45 HCM and 36 control subjects, we also performed exercise echocardiography. We measured peak velocity of early (E) and late (A) transmitral flow waves and peak early diastolic mitral-annular velocity (e'), at rest and during exercise.¹⁴ We also measured peak instantaneous LVOT gradient to rule out exercise-induced obstruction.¹⁵ Analysis of Doppler traces and echo images and measurements of wave amplitudes and chamber dimensions were performed by two independent blinded cardiologists with long-term experience in exercise echocardiography, and the two measured values were averaged to obtain each datapoint. Diastolic dysfunction at rest was defined when ≥ 2 of the following conditions were met¹⁴: (i) lateral $E/e' > 14$; (ii) lateral e' velocity < 10 cm/s; (iii) tricuspid regurgitation jet velocity > 2.8 m/s; (iv) left Atrial volume index > 34 mL/m². The worsening of diastolic function during exercise is considered when (i) lateral E/e' becomes > 14 , (ii) lateral e' gets < 10 cm/s, and (iii) tricuspid regurgitation velocity raises above 2.8 m/sec.¹⁴ Continuous clinical variables were expressed as means \pm SD and compared using Student's *t*-test, while categorical variables were reported as percentage and compared using chi-square test.

Results

In vitro study in isolated cardiomyocytes and trabeculae.

We performed force measurements in 9 NFNH-VD and 31 HCM trabeculae, as well as patch-clamp and/or calcium fluorescence measurements in 24 NFNH-VD and 47 HCM cardiomyocytes, isolated from 8 NFNH-VD and 24 HCM patients. As expected, cardiomyocytes from HCM patients were hypertrophic: in HCM vs. NFNH-VD cells, calculated cell volume was 38.9 ± 4.9 vs. 21.4 ± 4.0 pL ($P < 0.05$), and membrane capacitance was 184.8 ± 8.1 vs. 119.3 ± 6.2 pF ($P < 0.01$).

Effects of simulated exercise in human HCM myocardium

At baseline conditions, force twitches in intact HCM ventricular trabeculae show prolonged relaxation time and increased diastolic tension.² We simulated physiological exercise by combining β -adrenergic stimulation (ISO) with elevated pacing rates in HCM and control myocardium. Intact ventricular trabeculae were thus exposed to ISO while testing different stimulation rates (Figure 1A–C). Stimulation at 2 Hz combined with ISO (2Hz + ISO), with regards to the basal 1 Hz stimulation, produces a marked increase in twitch amplitude and a significant acceleration of twitch kinetics, and the entity of the variation was similar in NFNH-VD and HCM trabeculae (Figure 1A and B). However, the increase of diastolic tension while moving from 1 Hz to 2Hz + ISO was larger in HCM as compared with NFNH-VD trabeculae (Figure 1B). Moreover, despite the acceleration, twitch duration with ISO as still significantly longer in HCM vs. NFNH-VD trabeculae at any frequency investigated (Figure 1C). Overall, these observations suggest that in HCM ventricular myocardium β -adrenergic stimulation and high pacing rates, despite the marked contraction acceleration, cannot overcome the slower contractile kinetics of HCM myocardium, which is still slower than control myocardium and shows higher diastolic tension under maximal simulated exercise, suggesting stress-related diastolic abnormalities.

Effects of high frequency stimulation alone (2 Hz)

To assess the respective contributions of high pacing rates and β -adrenergic stimulation, we first compared the response of NFNH-VD and HCM ventricular myocytes and trabeculae to an increase of stimulation frequency from 0.5 Hz to 2 Hz (Figure 1D–G). APD shortened by a similar amount in NFNH-VD and HCM ventricular myocytes (Figure 1D, G), but was still longer in HCM vs. NFNH-VD myocytes at 2 Hz (APD90%: 338 ± 21 ms in NFNH-VD, vs. 449 ± 27 ms in HCM, $P < 0.01$). Ca^{2+} transients were also hastened by 2 Hz pacing in both groups (Figure 1E, G); again, they remained slower in HCM vs. NFNH-VD cells ($CaT50\% = 341 \pm 27$ ms in NFNH-VD vs. 417 ± 31 ms in HCM, $P < 0.01$). While Ca^{2+} -transient amplitude increased similarly at 2 Hz in both groups (Figure 1G), the increase of diastolic $[Ca^{2+}]$ was more pronounced in HCM myocytes. $[Ca^{2+}]_{diast}$ at 2 Hz was 112 ± 45 nM in NFNH-VD, vs. 277 ± 60 nM in HCM ($P < 0.01$ vs. NFNH-VD). Finally, raising stimulation frequency to 2 Hz in intact ventricular trabeculae led to an increase of both twitch amplitude and duration (Figure 1F and G) both in HCM and NFNH-VD muscles. At 2 Hz, however, twitch kinetics was still prolonged in HCM muscles vs. controls (For50% = 302 ± 21 ms in NFNH-VD vs. 363 ± 24 ms in HCM, $P < 0.05$). Finally, the increase in diastolic tension at 2 Hz was larger in HCM trabeculae than in control trabeculae (NFNH-VD: $+26 \pm 18\%$, HCM: $+68 \pm 36\%$, $P < 0.05$).^{2,10} Overall, the percentage shortening of APDs, Ca-transients and twitches when increasing HR was comparable in HCM and control myocardium. However, their kinetics, already prolonged at baseline,² remained significantly prolonged at fast pacing frequencies in HCM muscle, and the frequency-dependent increase of diastolic calcium and tension was more pronounced.

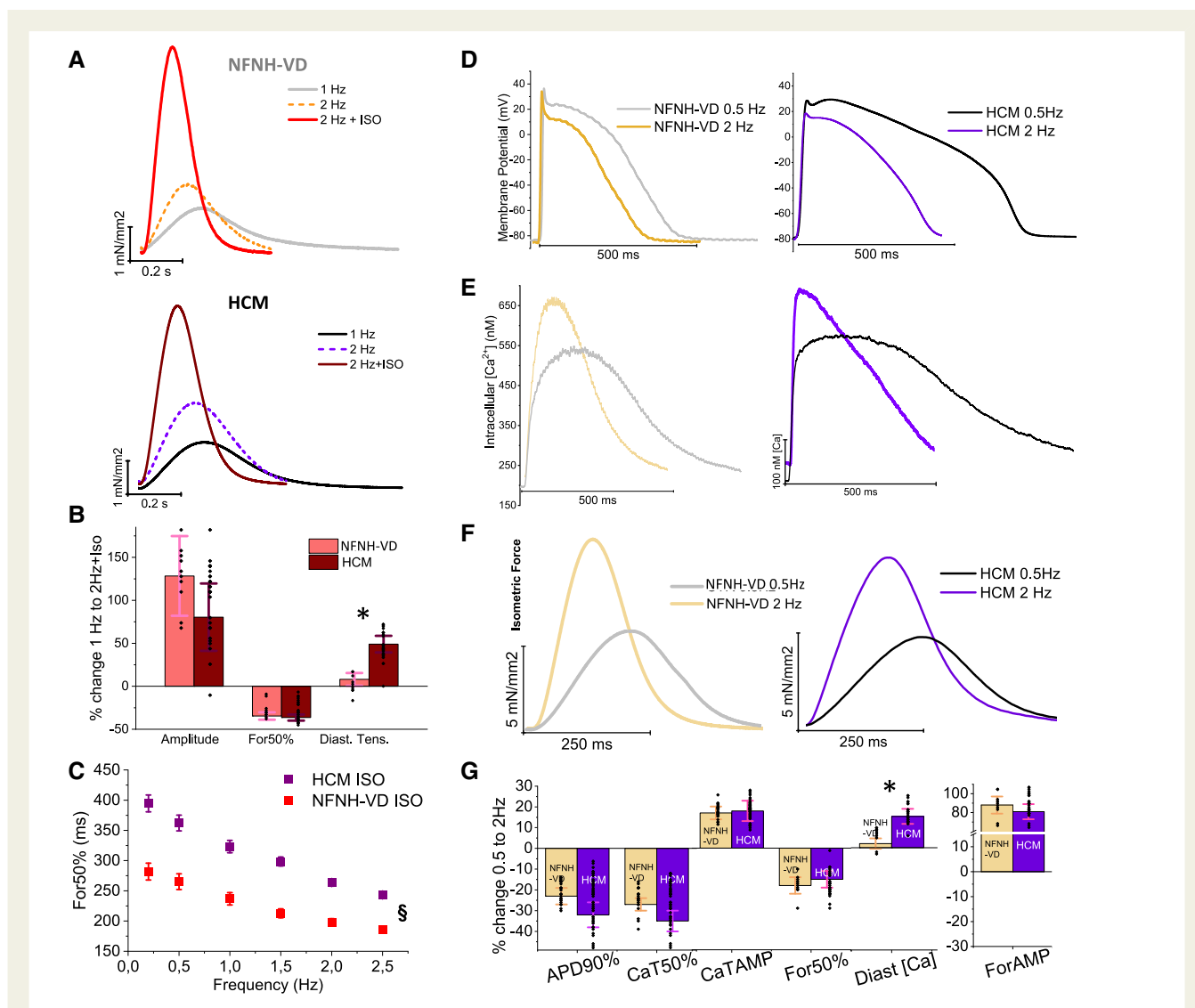


Figure 1 Effects of simulated exercise in trabeculae, effects of high stimulation frequency alone. (A) Representative superimposed twitches elicited during stimulation at 1, 2, and 2 Hz in the presence of isoproterenol, in ventricular trabeculae from non-failing non-hypertrophic valve-disease patients (used as controls, traces on top) and hypertrophic cardiomyopathy patients (bottom). (B) Percentage change of twitch amplitude and twitch duration to 50% relaxation (For50%) while going from 1 to 2 Hz + isoproterenol in non-failing non-hypertrophic valve-disease and hypertrophic cardiomyopathy trabeculae. Diast. Tens. = percentage variation of diastolic tension (expressed as a fraction of the amplitude of a steady state twitch). Average force results from nine CTR trabeculae (5 patients) and 31 hypertrophic cardiomyopathy trabeculae (21 hypertrophic cardiomyopathy patients). (C) For50% in the presence of isoproterenol in hypertrophic cardiomyopathy and non-failing non-hypertrophic valve-disease trabeculae. Average force results from nine non-failing non-hypertrophic valve-disease trabeculae (5 patients) and 31 hypertrophic cardiomyopathy trabeculae (21 hypertrophic cardiomyopathy patients). (D) Representative superimposed action potential traces elicited during stimulation at 0.5 and 2 Hz in control (left) and hypertrophic cardiomyopathy (right) cardiomyocytes. (E) Representative superimposed calcium transients recorded as in (A). (F) Representative superimposed isometric force twitches recorded in trabeculae. (G) Percentage change of different parameters when raising stimulation frequency from 0.5 to 2 Hz. From left: APD90% = action potential duration at 90% of repolarization; CaT50% = calcium transient duration from stimulus to 50% of decay; CaTAMP = amplitude of calcium transients (peak systolic-resting diastolic [Ca]); For50% = force twitch duration from onset to 50% of twitch relaxation; Diast [Ca] = diastolic Ca concentration at steady state; ForAMP = amplitude of force twitches. Means ± SEM from 24 control cardiomyocytes (8 patients) and 47 hypertrophic cardiomyopathy cardiomyocytes (24 hypertrophic cardiomyopathy patients). Average force results from nine non-failing non-hypertrophic valve-disease trabeculae (5 patients) and 31 hypertrophic cardiomyopathy trabeculae (21 hypertrophic cardiomyopathy patients). (B, D, G) In bar graphs, each black dot is a single myocyte or trabecula. Statistics: * = $P < 0.05$ vs. non-failing non-hypertrophic valve-disease, calculated using linear mixed models to encompass inter-patient variability. (C) § = $P < 0.05$ at all frequencies.

Effects of β -adrenergic stimulation alone (ISO 100 nM)

The effects of 100 nM ISO were assessed in HCM and NFNH-VD myocytes while recording APs and Ca^{2+} -transients during stimulation at 0.5 Hz (Figure 2). ISO led to APD shortening in NFNH-VD cardiomyocytes ($-16 \pm 3\%$), while it prolonged APD in HCM cardiomyocytes ($+23 \pm 8\%$, $P > 0.001$, Figure 2A, D). ISO increased the amplitude and accelerated the kinetics of Ca^{2+} transients in both NFNH-VD and HCM cells, by similar amounts (Figure 2B, D). Diastolic $[\text{Ca}]$ did not change in HCM cardiomyocytes when exposed to ISO, while it was slightly reduced in NFNH-VD cells (Figure 2B, D).

Additionally, the amplitude of force twitches in intact trabeculae increased by a similar extent in both NFNH-VD and HCM preparations in response to ISO (NFNH-VD: $+147 \pm 14\%$, HCM: $+153 \pm 13\%$). Twitch kinetics were similarly accelerated by ISO in HCM and NFNH-VD (For50%: $-24 \pm 4\%$ NFNH-VD, $-22 \pm 5\%$ HCM, Figure 2C and D). Nonetheless, despite the significant acceleration, twitch duration was still prolonged in HCM vs. NFNH-VD trabeculae even with ISO (NFNH-VD: 349 ± 29 ms, HCM: 458 ± 43 ms, $P < 0.01$). The abnormal electrical response to ISO in HCM myocytes was paralleled by an altered distribution of ion-currents. Indeed, we confirmed that the density of L-type Ca^{2+} -current (I_{CaL}) was slightly increased in HCM vs. NFNH-VD myocytes (at 0 mV, 6.6 ± 0.3 pA/pF in NFNH-VD, 7.3 ± 0.3 pA/pF in HCM, $P < 0.05$), while the density of delayed rectifier potassium current (I_{K}) was reduced (at 0 mV, 1.42 ± 0.36 pA/pF in NFNH-VD, vs. 0.55 ± 0.17 pA/pF in HCM, $P < 0.01$).^{2,7,8} Both currents are expected to increase in response to β -adrenergic stimulation. We observed that the amplitude of I_{CaL} similarly increased in response to ISO in NFNH-VD and HCM myocytes ($+34 \pm 12$ and $+30 \pm 10\%$, respectively, Figure 1E). However, the kinetics of I_{CaL} inactivation, already slower in HCM cells at baseline, was further prolonged by ISO (Figure 1E, F). Indeed, 50% I_{CaL} -decay (ICa50\%) in HCM myocytes was 74 ± 15 ms at baseline and increased to 94 ± 13 ms with ISO ($+22 \pm 6\%$); in NFNH-VD cells, ICa50\% was 43 ± 12 ms at baseline and 44 ± 14 ms with ISO ($P < 0.01$ vs. HCM). I_{K} response to ISO was also abnormal in HCM myocytes (1G-H); while I_{K} -density at 0 mV increased to 2.34 ± 0.47 pA/pF in NFNH-VD cells ($+69 \pm 10\%$), it only increased to 0.81 ± 0.17 pA/pF in HCM myocytes ($+39 \pm 7\%$). All in all, the abnormal distribution and function of β -adrenoceptor-responsive Ca^{2+} and K^{+} currents² cause an abnormal AP prolongation in response to β -adrenergic stimulation in HCM myocytes, while the acceleration of Ca^{2+} transients and force twitches appears to be normal, as it is linked with different mechanisms related with the sarcoplasmic reticulum and the myofilaments.³ Despite that, the kinetics of Ca-transients and twitches is still slower in HCM cells even under maximal β -adrenergic stimulation.

Mathematical modelling study

Next, we fitted the results obtained while changing stimulation frequency or adding ISO in single cells (Figure 1 and 2) into a validated mathematical model of HCM and control myocytes.^{6,7} The human ventricular cardiomyocyte model was adjusted to include the physiological and pathological responses of Ca and K currents observed in HCM and control cells, in order to fit the single-cell results obtained during β -adrenergic stimulation or upon increase of stimulation

frequency, taken separately (see Supplementary material online, Figures S1 and S2, respectively). The changes were then combined in our mathematical model to estimate the complete cellular effects of exercise (Figure 3). In short, adding β -adrenergic stimulation on top of heart-rate increase led to further shortening of APD and acceleration of Ca^{2+} -transient kinetics (Figure 3A), in line with the observations in trabeculae from HCM and NFNH-VD exposed to 'simulated exercise' (Figure 1A and B). On the other hand, the addition of β -adrenergic stimulation on top of 2 Hz pacing partially counteracted the acceleration of APs induced by HR elevation, ultimately resulting into a lesser shortening of APs and Ca^{2+} transients in HCM-cells (Figure 3B), in line with the observations obtained in HCM trabeculae (Figure 1C). All in all, mathematically modelled 'exercise' led to a comparable enhancement and acceleration of Ca^{2+} -transients and force in HCM vs. control cells, despite a smaller AP shortening, but the absolute duration of APs and Ca-transients was still longer in HCM modelled cells. (Figure 3C and D). As such observations were obtained in the model simply by changing the response of I_{K} and I_{Ca} to β -adrenergic stimulation in the HCM simulated cell, we concluded that the abnormal distribution of ion currents in HCM cells is the main responsible of the less efficient response to exercise of HCM cardiac muscle.

Clinical study

Exercise performance

Baseline patient characteristics are detailed in Table 1. Of note, the majority of HCM patients were in NYHA class I/II. Mean exercise time was similar between HCM patients and controls (11 ± 4 vs. 12 ± 4 min). However, maximum HR (expressed as percentage of predicted) was different (HCM: $77 \pm 9\%$ of predicted vs. controls: $89 \pm 9\%$, $P < 0.0001$). An abnormal blood pressure response was observed in 23 HCM patients. Peak exercise capacity was also lower in HCM, reflecting impaired exercise capacity (HCM: 6.5 ± 1.6 METs vs. controls: 9.8 ± 1.8 METs, $P < 0.05$) (Table 1). Exhaustion was the most common reason for interruption (90%) and there were no complications or arrhythmias during or after the test.

ECG changes with exercise

Compared to healthy individuals, HCM patients at peak exercise showed prolongation of RR interval, QRS duration, JTp interval and a Tp-e interval ($P < 0.001$ for all comparisons; Table 2). QTc increased during exercise by an average of 26 ± 14 ms from a baseline value of 437 ± 35 ms in HCM patients ($P < 0.0001$ vs. baseline, see Supplementary material online, Figure S3). QTc was significantly prolonged in HCM patients compared to controls both at rest and during exercise (QTc rest: HCM: 437 ± 35 vs. controls: 412 ± 23 - $P < 0.0001$ - and QTc exercise: HCM: 463 ± 49 vs. controls: 408 ± 43 - $P < 0.0001$ -) with significantly greater ΔQTc (HCM: $+27 \pm 52$ ms vs. controls: -4 ± 50 ms; $P < 0.0001$; Table 2). ΔQTc was inversely correlated with the ED interval duration at peak exercise (Figure 4A and B). The paradoxical QTc prolongation in HCM patients resulted in a marked reduction of ED intervals at peak exercise, with 34% of values < 100 ms (Figure 4A). These changes were much less significant in controls (Figure 4B). Interestingly, we observed an inverse linear relationship between ED interval at peak exercise and peak HR (Figure 4C and D). In patients with HCM, the ED/HR

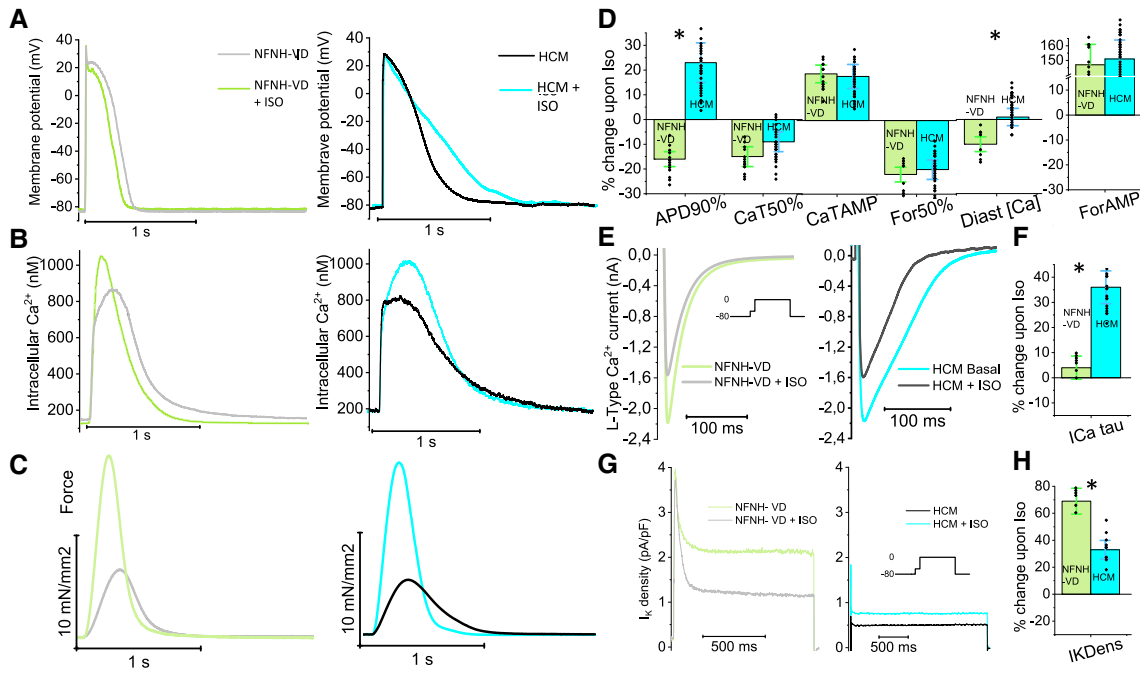


Figure 2 Effects of β -adrenergic stimulation alone in cardiomyocytes and trabeculae from non-failing non-hypertrophic valve-disease and hypertrophic cardiomyopathy patients. (A) Representative superimposed action potential traces elicited during 0.5 Hz stimulation in non-failing non-hypertrophic valve-disease (left) and hypertrophic cardiomyopathy (right) cardiomyocytes, in the absence and presence of isoproterenol 10^{-7} M. (B) Representative calcium transients, recorded as in (A). (C) Representative isometric force twitches at 0.5 Hz in intact trabeculae. (D) Percentage change of different parameters recorded in non-failing non-hypertrophic valve-disease and hypertrophic cardiomyopathy ventricular cardiomyocytes and trabeculae in response to isoproterenol. From left: APD90% = action potential duration at 90% of repolarization; CaT50% = calcium transient duration from stimulus to 50% of decay; CaTAMP = amplitude of calcium transients (peak systolic-resting diastolic [Ca]); For50% = force twitch duration from onset to 50% of twitch relaxation; Diast [Ca] = diastolic Ca concentration at steady state; ForAMP = amplitude of force twitches. Average cell data (Means \pm SEM) from 14 control cardiomyocytes (5 patients) and 30 hypertrophic cardiomyopathy cardiomyocytes (9 patients). Average force results from nine control trabeculae (5 patients) and 31 hypertrophic cardiomyopathy trabeculae (21 hypertrophic cardiomyopathy patients). (E) Representative superimposed L-type calcium current traces elicited at 0 mV from -80 mV resting potential in the absence and presence of isoproterenol, in non-failing non-hypertrophic valve-disease (left) and hypertrophic cardiomyopathy (right) cardiomyocytes. (F) Average change with isoproterenol of the τ (tau) of L-type Ca^{2+} -current decay calculated after fitting the decay of the current with an exponential (Ica tau). Means \pm SEM from seven control myocytes (3 patients) and 15 hypertrophic cardiomyopathy myocytes (6 patients). (G) Representative delayed rectifier potassium current traces elicited at 0 mV from -80 mV resting potential in control (left) and hypertrophic cardiomyopathy (right) cardiomyocytes, in the absence and presence of isoproterenol. (H) Average change in the density of potassium current at 0 mV upon isoproterenol. Means \pm SEM from six control myocytes (3 patients) and 10 hypertrophic cardiomyopathy myocytes (3 patients). (D, F–H) In bar graphs, each black dot is a single myocyte or trabecula. **Statistics:** * = $P < 0.05$ vs. non-failing non-hypertrophic valve-disease, calculated using linear mixed models to encompass inter-patient variability.

relationship was steeper than in controls (slope: -2.81 in HCM vs. -2.11 in controls; [Figure 4](#)). In HCM patients, excessive shortening of the electrical diastole due to QTc prolongation appeared to limit the increase in HR during exercise. Interestingly, metabolic equivalents (METs) at peak exercise in HCM patients were directly correlated with the duration of electrical diastole, and were inversely correlated with peak HR (see [Supplementary material online, Figure S4](#)).

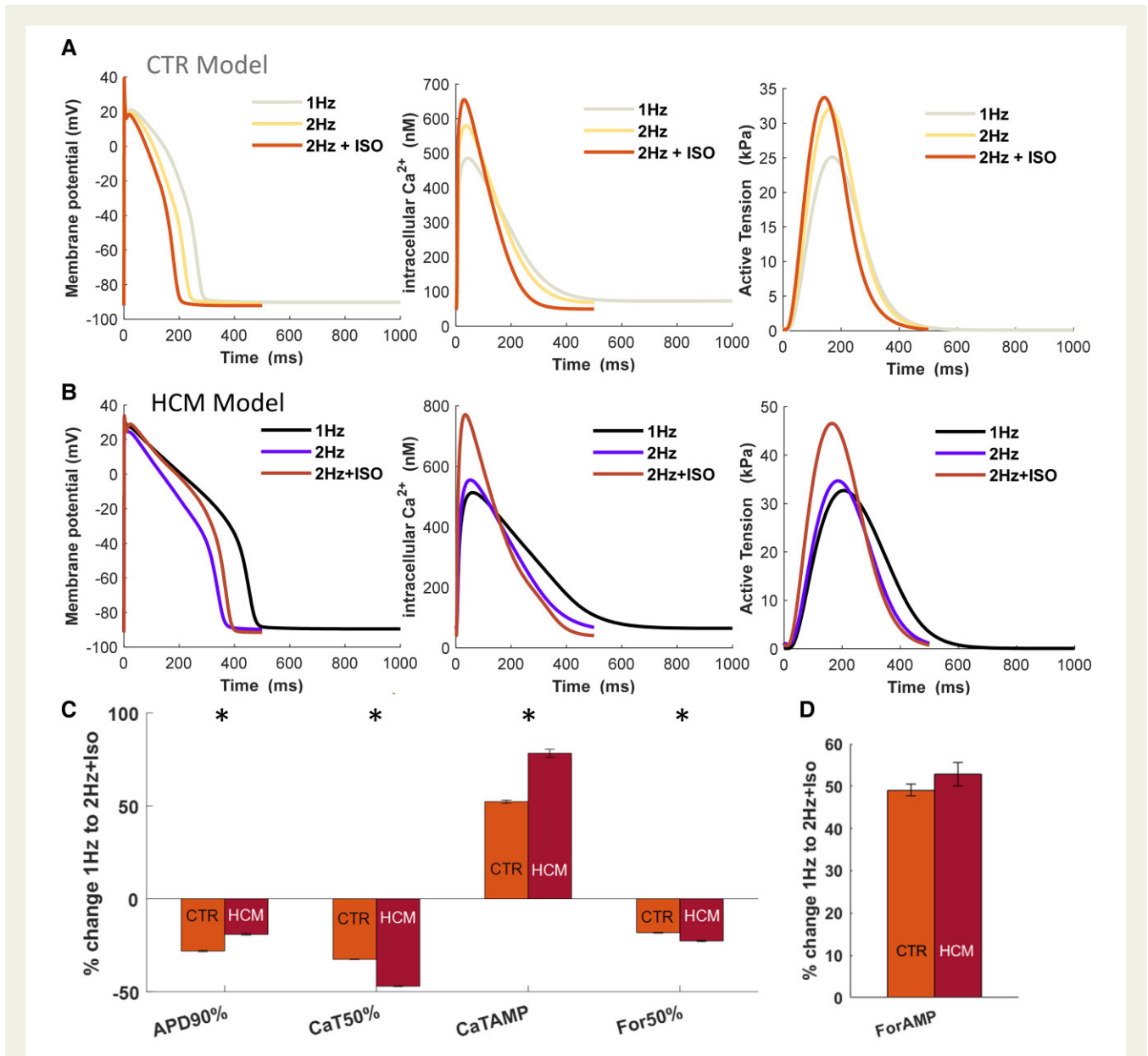
Echocardiographic findings at rest and during exercise

At baseline, HCM patients showed impaired diastolic function. Specifically, at rest HCM patients showed increased atrial dimensions, lower e' lateral velocity and higher LV filling pressures

compared to controls ([Table 2](#)). Moreover, HCM patients showed a higher LVEF. Both E and e' lateral wave velocities progressively increased during exercise in healthy subjects, and a physiological diastolic reserve was demonstrated by persistence of normal E/ e' values during effort (see [Supplementary material online, Figure S3](#)). E and e' increased with exercise also in HCM patients; however, E/ e' ratio increased with exercise, reflecting reduced diastolic reserve ([Table 2](#)).

QTc prolongation (>30 ms) is associated with worsening of diastolic function

Among HCM patients at rest, 33 of 45 (73%) had normal diastole, while 12 of 45 (27%) had diastolic dysfunction. At peak exercise, diastolic function remained normal in 21 of 45 (47%) patients, while



diastolic function worsened in 12 of 45 patients (27%). In the remaining 12 patients, diastolic dysfunction persisted during exercise.

To attempt a correlation between changes of ECG and diastolic function during exercise, we divided patients based on ΔQTc . We distinguished patients where ΔQTc was >30 ms during exercise ('with exercise-induced QTc increase'; $n = 24/45$; 53%) and patients where exercise QTc remained stable or increased by <30 ms

('without exercise-induced QTc increase'; $n = 21$; 47%) (Figure 5). Interestingly, E/e' values at rest were similar in patients belonging to the two subgroups (Figure 5A). Nonetheless, we observed a strong correlation between QTc increase and worsening of diastolic function during exercise. Notably, 10 of the 12 patients with worsening diastolic function during exercise had exercise-Induced QTc increase; conversely, only 8 of 22 patients with persistently normal

Table 2 Electrocardiogram features and echocardiographic data at rest and during exercise

ECG data								
	HCM (n = 178)			Control (n = 81)			HCM vs. CTR (unpaired P values)	
	At rest	At peak exercise	P (paired)	At rest	At peak exercise	P (paired)	At rest	At peak exercise
RR (ms)	942 ± 169	473 ± 96	<0.0001	865 ± 172	437 ± 79	<0.0001	<0.0001	0.003
QRS (ms)	90 ± 14	93 ± 14	>0.05	87 ± 10	85 ± 10	>0.05	<0.0001	<0.0001
JTp (ms)	239 ± 39	140 ± 35	<0.0001	215 ± 38	119 ± 20	<0.0001	<0.0001	<0.0001
Tp-e (ms)	92 ± 19	84 ± 23	<0.01	77 ± 12	65 ± 15	<0.001	<0.0001	<0.0001
QTc (ms)	437 ± 35	463 ± 49	<0.001	412 ± 23	408 ± 43	>0.05	<0.0001	<0.0001
ΔQTc	+27 ± 52			-4 ± 50			<0.0001	

Echocardiographic data								
	HCM (n = 45)			Control (n = 36)			HCM vs. CTR (unpaired P values)	
	At rest	At peak exercise	P (paired)	At rest	At peak exercise	P (paired)	At rest	At peak exercise
Maximal LV thickness mm	18 ± 4	NA	NA	8 ± 2	N/A	N/A	<0.0001	N/A
LA diameter mm	41 ± 6	NA	NA	30 ± 5	N/A	N/A	<0.0001	N/A
LVEDV index ml/m ³	49 ± 8	40 ± 7	<0.01	52 ± 8	45 ± 8	<0.01	>0.05	<0.05
LVEF (%)	69 ± 6	71 ± 7	>0.05	62 ± 7	69 ± 7	<0.05	<0.001	>0.05
E wave (cm/s)	66 ± 11	128 ± 15	<0.0001	78 ± 10	110 ± 12	<0.0001	<0.01	<0.05
A wave (cm/s)	61 ± 9	111 ± 12	<0.0001	62 ± 10	79 ± 13	<0.001	>0.05	<0.001
E/A ratio	1.18 ± 0.8	1.21 ± 0.8	>0.05	1.29 ± 0.6	1.37 ± 0.6	>0.05	<0.01	<0.001
e' lateral (cm/s)	9.4 ± 2.1	16.1 ± 3.2	<0.01	13.2 ± 2.8	18.8 ± 3.2	<0.01	<0.0001	<0.05
E/e' lateral	7.6 ± 2.3	9.8 ± 2.4	<0.05	5.7 ± 1.8	6.0 ± 1.6	>0.05	<0.05	<0.001
LVOT gradient (mmHg)	9 ± 6	19 ± 6	<0.05	5 ± 3	7 ± 4	>0.05	>0.05	<0.01

JTp, time from end of QRS to peak T wave; Tp-e, Tpeak-Tend interval; LV, left ventricular; LVOT, LV outflow tract; LA, left atrial; LVEDV, LV end diastolic volume; LVEF, LV ejection fraction.

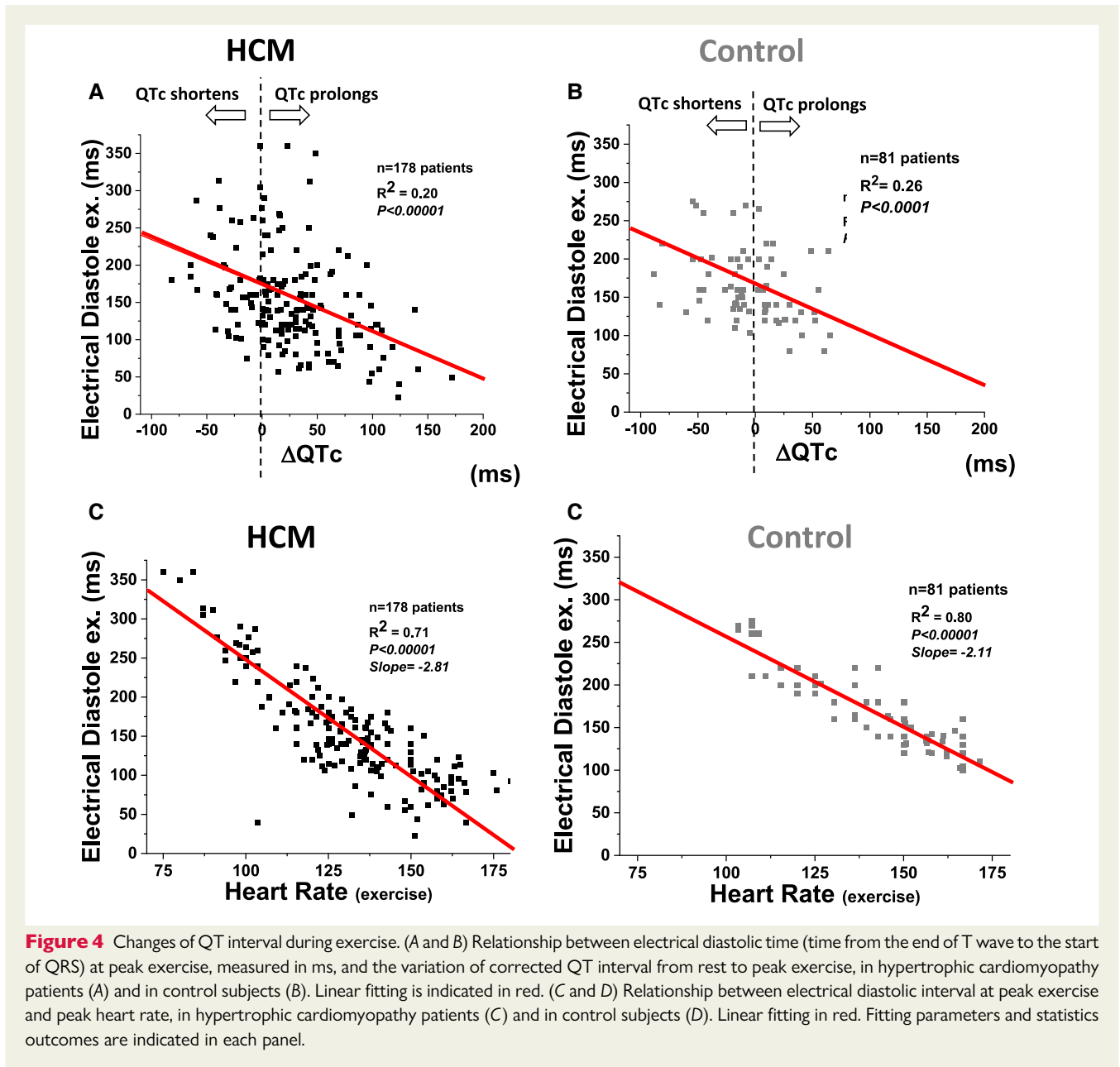
P calculated with Student's t-test (paired groups for exercise vs. rest comparisons; unpaired groups for HCM vs. CTR comparisons).

diastole had exercise-induced QTc increase ($P = 0.015$ at chi-square test). Consistently, average E/e' ratio at peak exercise was significantly higher among the patients with exercise-induced QTc increase (Figure 5B). Indeed, E/e' increased more substantially in patients where we observed a QTc prolongation during exercise (Figure 5C). Finally, we observed that the worsening of diastolic function in patients with exercise-induced QTc prolongation was associated with chronotropic insufficiency, as the maximal HR at peak exercise was significantly lower (Figure 5D).

Discussion

HCM is universally defined as a 'diastolic disease' and the evaluation of LV diastolic (dys) function is at the core of its clinical management and prediction of outcome.¹⁶ While diastolic impairment is considered a major determinant of exercise limitation in HCM, little is known regarding the behaviour of diastolic reserve on effort. Here we assessed the relation between ED time by ECG and mechanical diastolic function by tissue-Doppler echocardiography during stress.

Notably, the absolute QT interval shortened by a lesser extent in HCM vs. control patients on effort (Figure 4, Table 2), consistent with the lesser shortening of APs in HCM cardiomyocytes. The direct implication of an insufficient shortening of the QT interval is the reduction of the electrical diastole.¹⁷ The duration of the electrical diastole at peak exercise is inversely proportional to QTc prolongation from rest (Figure 4). The shortening of ED appears to contribute to the limited maximal frequency achieved during exercise by HCM patients: the slope of the ED/HR relationship was steeper in HCM vs. control patients, reaching a virtual value of 0 ms at about 180 bpm. As a zero ED interval is not physiologically attainable, such relationship suggests that exercise-related increase in HR is limited in HCM hearts and that, for very short ED intervals, the LV may be unable to relax properly during diastole (Central Illustration). Patients in whom the QTc prolonged by more than 30 ms during exercise, resulting in a short ED interval, despite a similar diastolic function at rest (Figure 5A), were more likely to experience worsening of diastolic function (increased E/e'; Figure 5B) during exercise and impaired performance with lower peak HR (Figure 4 and Figure 5D). Conversely, patients in whom the QTc remained relatively constant had a preserved diastolic function during exercise and reached a higher peak HR.



Response of HCM myocardium to β -adrenergic stimulation and high stimulation frequencies

We studied the fine mechanisms underlying the response of CTR and HCM ventricular myocardium to β -adrenergic stimulation and high-frequency pacing (Figures 1 and 2). Although the two phenomena occur simultaneously during physiological exercise, we separated them to dissect their relative contributions.

Activation of β -adrenergic receptors is known to lead to an increase of both L-Type Ca^{2+} current (I_{CaL}) and slow delayed rectifier I_{K} , via protein kinase A (PKA)-mediated phosphorylation of Cav1.2 and Kv7.1 channels, respectively. Consistently, we observed that the amplitude of I_{CaL} and I_{K} are increased by ISO in both HCM and control cardiomyocytes (Figure 1E–H). However, the baseline density

of I_{CaL} is slightly increased in HCM vs. control cells, while the density of I_{K} is severely reduced in HCM ventricular cardiomyocytes, due to previously reported changes of mRNA expression, including a reduction of Kv7.1². In HCM myocytes, the relative increase of I_{K} in response to ISO is lower with respect to control cells (Figure 4H), rendering the I_{K} deficiency of HCM myocytes even more pronounced under β -stimulation, when I_{K} density is approximately 1/3 of that of controls (Figure 1G). Conversely, while the density of I_{CaL} is physiologically increased by ISO in HCM myocytes (Figure 1E), the kinetics of current inactivation, already slower at baseline, is further delayed by ISO in HCM cells (Figure 1F). In HCM cells, the abnormal effects of β -stimulation on I_{CaL} and I_{K} ultimately lead to delayed repolarization (Figure 6). The mechanical response of HCM myocardium to β -agonists, however, was maintained, in that ISO showed both positive inotropic and lusitropic (i.e. acceleration of relaxation) effects in

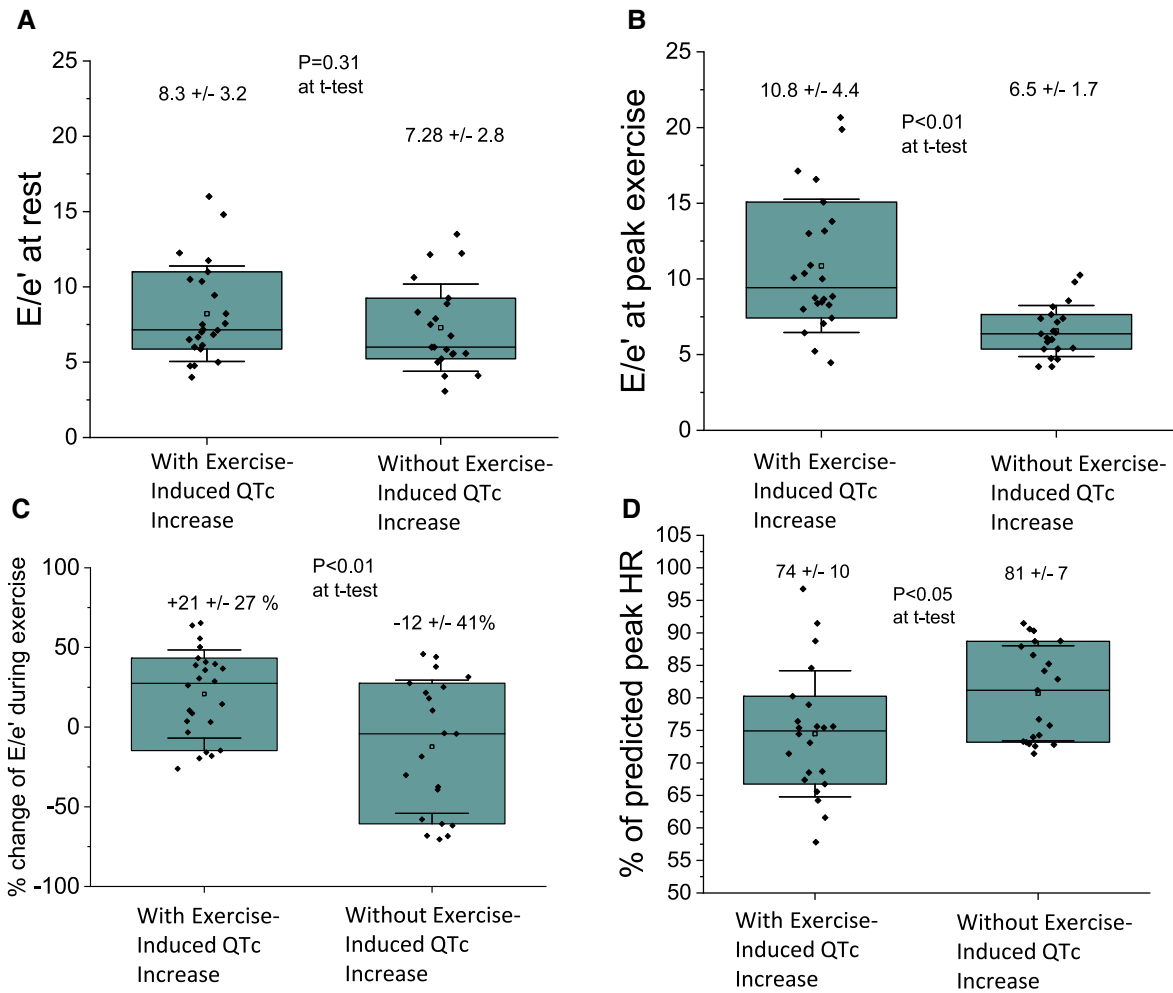


Figure 5 Diastolic function, heart rate, and QT variations. E/e' ratio calculated at rest (A) and at peak exercise (B) in the 24 hypertrophic cardiomyopathy patients in whom QTc prolongs by more than 30 ms from rest to exercise ('with exercise-induced QTc increase') and in the remaining 21 patients with no change ('without exercise-induced QTc increase'). (C) Percentage change of E/e' during exercise in the two subgroup of hypertrophic cardiomyopathy patients. (D) Percentage of predicted peak heart rate in the two groups of hypertrophic cardiomyopathy patients. Each dot is a patient. Each box plot shows 20–80% range (box height), average value (empty square inside the box), median value (horizontal line inside the box), and standard deviation (black whiskers). Statistics: unpaired t-test.

HCM trabeculae (Figure 1C and D). These effects are mediated by the PKA-phosphorylation of excitation-contraction coupling proteins, i.e. ryanodine receptors (RyR2) and phospholamban, potentiating Ca^{2+} -release from the sarcoplasmic-reticulum and hastening Ca^{2+} reuptake (Figures 2 and 6). Such effects appear to be normally occurring in HCM cardiomyocytes, although the duration of Ca^{2+} transients, already slower at baseline^{2,7,10} is still longer in HCM cells during β -stimulation. In addition, the decrease of myofilament Ca^{2+} -sensitivity by PKA-mediated Troponin-I phosphorylation helps speeding up muscle relaxation. Nonetheless, twitch duration with ISO was still longer in HCM vs. NFNH-VD samples (Figure 1C and D).

The increase of pacing rate (0.5-to-2 Hz) led to similar changes in HCM and control myocardium, suggesting a preserved response to high frequencies. Action potentials, Ca-transients and force twitches shortened by a comparable amount in HCM and

NFNH-VD cells when increasing frequency from 0.5 to 2 Hz; nonetheless, they were all still significantly longer in HCM vs. control myocardium at 2 Hz. Moreover, diastolic [Ca] and diastolic tension increased by larger amounts in HCM vs. NFNH-VD when increasing frequency, suggesting a worsening of diastolic function.

The combination of β -stimulation and high frequencies was tested in trabeculae (Figure 1). The opposite effects of β -stimulation and high frequencies on APD caused a lesser shortening of APs in HCM vs. control muscle, while Ca^{2+} -transients and twitches hastened by comparable amounts. Despite the acceleration of contraction and relaxation in HCM ventricular muscle with simulated exercise, Ca^{2+} transients and twitches were still quantitatively slower in HCM myocardium as compared with control muscle. Moreover, the increase of diastolic tension when simultaneously adding β -stimulation and high-frequencies was significantly larger in

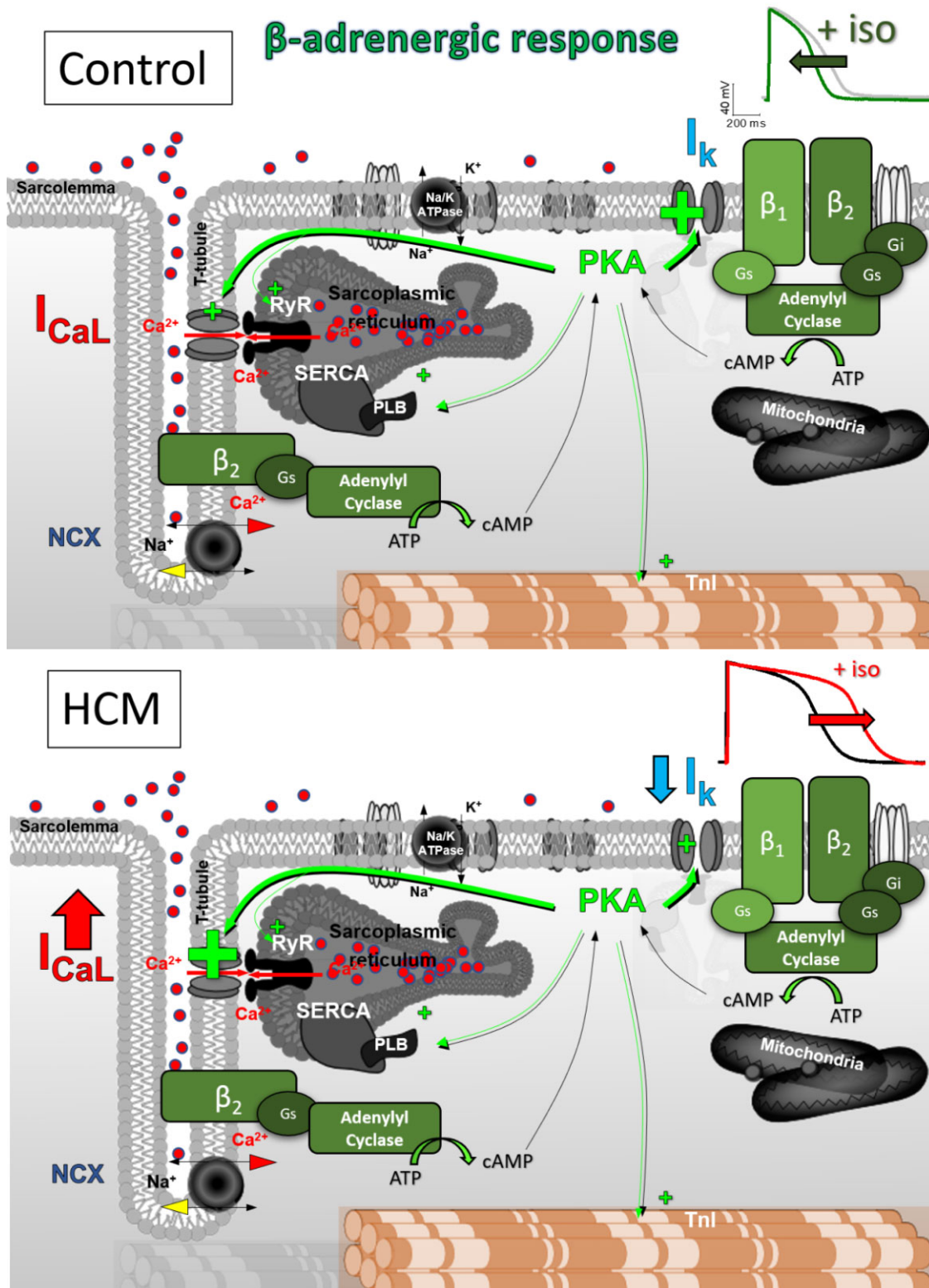


Figure 6 Altered adrenergic response in hypertrophic cardiomyopathy myocytes. Scheme depicting the altered response of hypertrophic cardiomyopathy cardiomyocytes to beta-adrenergic stimulation. B-adrenergic receptors activate adenylyl cyclase through G_s subunits, which in turn generate cyclic AMP. cyclic AMP activates protein kinase A, which phosphorylates a number of intracellular targets (green plus symbols), including calcium and potassium channels. In hypertrophic cardiomyopathy cells, at variance from controls, the net effect of β stimulation in hypertrophic cardiomyopathy cells is an increase of depolarizing currents driven by the heightened L-type Ca²⁺-current, ultimately resulting in a longer action potential.

HCM trabeculae, suggesting a possible impairment of diastolic function during exercise. Mathematical modelling confirmed that the reduction of K^+ currents and the abnormal response of ICa to β -stimulation were important contributors to the abnormal electrical response of HCM muscle to exercise, ultimately leading to prolonged contraction at peak stress.

Limitations

- (i) The *in vitro* study was performed in samples from severely obstructive patients undergoing myectomy, while the clinical study was conducted in non-obstructive patients with mild or no symptoms. Performing exercise stress tests on severely obstructive patients enlisted for myectomy would have made it impossible to identify changes of diastolic function during exercise, as exercise performance in those patients is crippled by the increase of LVOT gradients, preventing them from reaching the minimal acceptable levels of performance. Alternatively, we could have performed stress tests on the 23 myectomy patients after the operation. However, myectomy patients invariably develop a severe left bundle branch block, which would render all QT measurements unusable. Having no feasible alternatives, we chose to perform our clinical study on a subpopulation of HCM patients with very mild disease, where all the observed abnormalities occurring during exercise tests are likely to be a direct consequence of HCM-related changes of the electrical and mechanical function of cardiomyocytes, in the absence of other confounding factors that may appear in subjects with a more severe disease (changes of the neurohormonal status, effects of drug therapy, consequences of severe myocardial fibrosis).
- (ii) In our *in vitro* and mathematical modelling experiments, we attempted to mimic exercise by combining β -adrenergic stimulation with high frequency stimulation. During physiological exercise, increased venous return enhances diastolic filling and increases end-diastolic LV volume, leading to length-dependent activation of myofilament ('Frank-Starling' mechanism). Our experiments and models do not consider a possible increase of end-diastolic sarcomere length during exercise, and cannot mimic the exercise-related changes of hemodynamic conditions.
- (iii) Chronic Beta-blocker treatment in obstructive patients who underwent myectomy might have influenced the response of their myocardial cells to ISO. Even though all patients stopped receiving β -blockers at least 2 days before the intervention (> 3 drug half-lives), long term effects on receptor expression might have occurred. Nonetheless, we do not believe that this may have influenced the differences we observed between HCM and control samples, as 75% of patients belonging to our non-failing non-hypertrophic cohort were also on β -blocker treatment prior to the intervention (see [Supplementary material online, Table S1](#)).

Conclusions

Reduced exercise tolerance is the most prevalent symptom in HCM, with a significant impact on patients' quality of life. In some patients without obstruction, exercise intolerance is linked to diastolic dysfunction, albeit a detailed assessment of diastolic function during exercise is rarely performed during standard clinical assessments. By

performing echocardiography tests on a bicycle ergometer, we here observed that diastolic function worsens during exercise in a significant percentage of non-obstructive HCM patients. Interestingly, the worsening of diastolic function during exercise in HCM patients is associated with a prolongation of the frequency-corrected QT interval (QTc) when going from rest to peak exercise.

Using cardiomyocytes isolated from ventricular samples of surgical HCM, we observed that action potentials were insufficiently shortened by simulated exercise *in vitro* (β -adrenergic stimulation and high pacing rates). This abnormal response was determined by changes of the expression and properties of Ca^{2+} and K^+ currents in HCM myocardium. In HCM patients, this translates into exercise-induced QTc prolongation and impaired diastolic reserve, contributing to reduced exercise tolerance. Moreover, the increased intracellular diastolic $[Ca^{2+}]$ concentration during exercise in HCM cells underlies a slower myocardial relaxation and an increased diastolic tension, with possible consequences in terms of myocardial ischaemia. As cardiomyocyte electrical abnormalities are amplified during 'exercise', they may have significant implications for the development of arrhythmias during stress or physical exertion in patients. All in all, our studies support the idea that electrical myocardial abnormalities contribute to exercise-related worsening of diastole and stress-induced angina in patients with HCM, representing a promising therapeutic target for the reduction of exertional symptoms in non-obstructive patients. In particular, drugs that accelerate repolarization by reducing depolarizing currents active during the plateau of the action potential (for example, ranolazine blocking I_{NaL}) may reduce the abnormal electrical response to exercise, contributing to improve the exercise capacity of patients.

Lead author biography



Raffaele Coppini: He is a clinician scientist with a PhD in preclinical cardiovascular pharmacology and a medical specialization in clinical pharmacology. After a position as a staff researcher in the laboratory of Elisabetta Cerbai, he is currently a tenure-track assistant professor and directs a laboratory of cardiovascular pharmacology and cardiac cellular electrophysiology in the NeuroFarBa

Department of the University of Florence. He is best known for his work on the pathophysiology of hypertrophic cardiomyopathy (HCM) and for studies aimed at testing novel therapeutic approaches for the treatment of HCM, performed in human cardiomyocytes from cardiac surgery samples, mouse models or iPSC-derived cardiomyocytes.

Author contributions

R.C. devised the *in vitro* protocol, performed single cell experiments, analyzed the data and wrote the paper. M.B. devised the clinical protocol, performed clinical study activities, collected and analyzed clinical data and critically reviewed the manuscript. R.D. devised

the modelling study, programmed the mathematical model and performed modelling experiments. A.B.-O. contributed to the model and critically reviewed the manuscript. C.F. performed experiments on human trabeculae and critically reviewed the manuscript. G.V. and J.M.P. contributed to trabeculae experiments on human samples. L.S. performed single cell experiments on cardiomyocytes from human samples. A.A., M.B., and F.M. performed activities related to the clinical study in HCM patients. N.M., E.C., and C.P. edited and critically reviewed the manuscript. P.S. provided cardiac samples and surgical patient data and critically reviewed the manuscript. I.O. devised the study, coordinated the different parts of the study, and contributed to write the paper.

Ethical standards

All protocols involving patients were approved by the ethical committee of Careggi University-Hospital (2006/0024713; renewed May 2009) and were conducted in accordance with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All subjects recruited for this study gave their informed consent prior to their inclusion in the study.

Data availability

The raw results of the study are available upon request to the authors.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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