RAPID REPORT

The familial hypertrophic cardiomyopathy-associated myosin mutation R403Q accelerates tension generation and relaxation of human cardiac myofibrils

Alexandra Belus[1,2], Nicoletta Piroddi[1,2], Beatrice Scellini[1,2], Chiara Tesi[1,2], Giulia D, Amati[3], Francesca Girolami[4], Magdi Yacoub[5,6], Franco Cecchi[5,6], Iacopo Olivotto[6] and Corrado Poggesi[1,2]

[1] *Department of Physiology, University of Florence, Florence, Italy*
[2] *Center of Molecular Medicine (CIMMBA), Florence, Italy*
[3] *Department of Experimental Medicine and Pathology, La Sapienza University, Rome, Italy*
[4] *Department of Genetic Diagnosis, Careggi University Hospital, Florence, Italy*
[5] *Department of Medical and Surgical Critical Care, University of Florence, Florence, Italy*
[6] *Regional Referral Center for Myocardial Diseases, Careggi University Hospital, Florence, Italy*

The R403Q mutation in β-myosin heavy chain was the first mutation to be identified as responsible for familial hypertrophic cardiomyopathy (FHC). In spite of extensive work on the functional sequelae of this mutation, the mechanism by which the mutant protein causes the disease has not been definitely identified. Here we directly compare contraction and relaxation mechanics of single myofibrils from left ventricular samples of one patient carrying the R403Q mutation to those from a healthy control heart. Tension generation and relaxation following sudden increase and decrease in $[Ca^{2+}]$ were much faster in the R403Q myofibrils with relaxation rates being the most affected parameters. The results show that the R403Q mutation leads to an apparent gain of protein function but a greater energetic cost of tension generation. Increased energy cost of tension generation may be central to the FHC disease process, help explain some unresolved clinical observations, and carry significant therapeutic implications.

(Received 28 April 2008; accepted after revision 14 June 2008; first published online 19 June 2008)
Corresponding author C. Poggesi: Dipartimento di Scienze Fisiologiche, Università di Firenze, Viale Morgagni 63, 50134 Firenze, Italia. Email: corrado.poggesi@unifi.it

The R403Q mutation in the β-myosin heavy chain was the first mutation to be identified as responsible for familial hypertrophic cardiomyopathy (FHC) (Geisterfer-Lowrance *et al.* 1990), a primary disease of the cardiac sarcomere that is the most commonly identified cause of cardiac sudden death in young people. The functional sequelae of the R403Q mutation have been extensively investigated using a variety of experimental models and approaches (Cuda *et al.* 1993; Lankford *et al.* 1995; Sata & Ikebe, 1996; Geisterfer-Lowrance *et al.* 1996; Marian *et al.* 1999; Tyska *et al.* 2000; Lowey, 2002; Keller *et al.* 2004) but the cardiac sarcomeres of affected individuals have never been directly examined. Here we compare contraction and relaxation of left ventricular myofibrils from one patient carrying the R403Q mutation to those from a healthy control heart. To investigate sarcomere mechanics we use previously published techniques to measure and control the force and length of single myofibrils activated and relaxed by fast solution switching (Tesi *et al.* 2002; Piroddi *et al.* 2007). One advantage of this approach was that we could probe the acto-myosin transduction cycle while keeping the native structured sarcomere environment of the mutant protein. Preliminary report of this work has been published in abstract form (Belus *et al.* 2007).

This paper has online supplemental material.

Methods

Patients

The investigation conforms with the principles outlined in the *Declaration of Helsinki* and had been approved by the local Ethics Committee. Informed consent was given for both mutational analysis and mechanical experiments.

A 24-year-old man, with a severe family history of premature cardiac death, diagnosed at 13 with FHC, and

 DOI: 10.1113/jphysiol.2008.155952

now with progressive worsening of dyspnoea on effort was referred to our institution (see online Supplemental file and figure). Mutational analysis of the myofilament protein-encoding genes associated with FHC identified a missense R403Q mutation in the gene coding for β-myosin heavy chain (*MYH7*). As the patient showed significant resting outflow obstruction (peak gradient estimated at rest by continuous wave Doppler was 85 mmHg), he underwent extended surgical myectomy. Small samples from the myectomy were used for myofibril isolation.

Control myofibrils were obtained from samples of the left side of the interventricular septum of a female healthy donor (24 years) heart that had not been transplanted for technical reasons. Both R403Q and control samples were transported in cold cardioplegic solution and, on arrival in the laboratory, immediately frozen in liquid nitrogen, stored at -80°C, then used for myofibril isolation within the following few weeks. Previously reported data (Piroddi *et al.* 2007) from myofibrils of non-failing, non-hypertrophic left ventricular samples of patients undergoing corrective cardiac surgery could also be used as controls.

Myofibril preparation and apparatus

Ventricular specimens were cut into thin strips under a stereomicroscope in ice-cold rigor solution containing (mM): NaCl 132, KCl 5, $MgCl_2$ 1, Tris 10, EGTA 5 (pH 7.1). The strips were incubated for 3 h in the same solution with added 1% Triton-X 100. Triton was then removed and the strips were homogenized in rigor solution to produce myofibril suspensions.

In the present experiments we used previously published techniques (Tesi *et al.* 2002; Piroddi *et al.* 2007). Briefly, a small volume of the myofibril suspension was transferred to a temperature-controlled chamber (15°C) filled with relaxing solution (pCa 8.0) and mounted on an inverted microscope. Selected preparations (single myofibrils or bundles of few myofibrils, 25–80 μm long, 1–4 μm wide) were mounted horizontally between two glass microtools. One tool was connected to a length-control motor that could produce rapid (< 1 ms) length changes. The second tool was a calibrated cantilevered force probe (2–6 nm nN^{-1}; frequency response 2–5 kHz). Force was measured from the deflection of the image of the force probe projected on a split photodiode. Average sarcomere length and myofibril diameter were measured from video images (ca 1800 ×).

The initial sarcomere length of the preparations was set around 2.2 μm. Myofibrils were activated and relaxed by rapidly translating the interface between two flowing streams of different pCa solutions across the length of the preparation. The solution change took place with a time constant of 2–3 ms and was complete in less than 5 ms.

Activating and relaxing solutions, calculated as previously described (Tesi *et al.* 2002), were at pH 7.00 and contained 10 mM total EGTA (CaEGTA/EGTA ratio set to obtain pCa 8.0 and 4.5), 5 mM MgATP, 1 mM free Mg^{2+}, 10 mM Mops, propionate and sulphate to adjust the final solution to an ionic strength of 200 mM and monovalent cation concentration of 155 mM. Creatine phosphate (CP; 10 mM) and creatine kinase (200 unit ml^{-1}) were added to all solutions. Contaminant inorganic phosphate (P_i) from spontaneous breakdown of MgATP and CP was reduced to less than 5 μM by a P_i scavenging system (purine-nucleoside-phosphorylase with substrate 7-methyl-guanosine) (Tesi *et al.* 2002). All solutions to which the samples and myofibrils were exposed contained a cocktail of protease inhibitors including leupeptin (10 μM), pepstatin (5 μM), PMSF (200 μM) and E64 (10 μM), as well as NaN_3 (500 μM) and DTE (2 mM).

Results

Figure 1 shows, for both control donor and R403Q myofibrils, representative traces of tension responses to maximal Ca^{2+} activation by fast solution switching; average data for both myofibril groups are shown in Table 1 together with data previously reported for myofibrils taken from left ventricular samples of control (non-failing, non-hypertrophic) patients (Piroddi *et al.* 2007). Control data from the age-matched donor heart were undistinguishable from the previously reported controls; as shown by Piroddi *et al.* (2007), in spite of some variability, myofibril mechanical and kinetic parameters did not differ significantly among myofibril populations taken from different control samples.

In the R403Q myofibrils, maximal isometric tension (P_0) was lower ($P < 0.01$ *versus* control patients) or tended to be lower ($P < 0.1$ *versus* control donor) whereas kinetics of tension development (k_{ACT}, as well as k_{TR}), were markedly faster compared to both control groups (Table 1 and Fig. 1*B*), indicative of faster cross-bridge turnover rate. In all myofibril populations, the time course of Ca^{2+}-activated tension development was mono-exponential; the activation rate constant (k_{ACT}) was the same as k_{TR}. The latter is the rate constant of tension redevelopment following large mechanical perturbations, applied to the myofibril during steady Ca^{2+} activation, aimed at detaching most of force-generating crossbridges (Brenner, 1988). The similarity between k_{ACT} and k_{TR} shows that k_{ACT} is not limited by the rate with which thin filaments are switched on by Ca^{2+}, but rather reflects isometric crossbridge turnover rates (i.e. the sum of the apparent rates with which crossbridges enter and leave their force-generating states).

Active tension fully relaxed with step reduction of $[Ca^{2+}]$ below the contraction threshold (Fig. 1*A*). The

kinetics of tension relaxation in control donor and R403Q myofibrils are shown on a faster time scale in Fig. 1*C*. As previously described (Piroddi *et al.* 2007), the time course of force relaxation in human cardiac myofibrils was biphasic, starting with a slow, seemingly linear, force decay followed, after a 'shoulder', by a fast, exponential, relaxation phase. It has been shown (Poggesi *et al.* 2005) that the slow linear force decay occurs while sarcomeres are isometric and its rate constant (slow k_{REL}) is predominantly the apparent rate with which attached crossbridges leave force-generating states; the fast exponential phase follows the 'give' of a few sarcomeres and is dominated by intersarcomere dynamics. Relaxation kinetics were much faster in the R403Q myofibrils than in the control myofibrils (Table 1 and Fig. 1*C* and *D*). The rate constant of the fast relaxation phase (fast k_{REL}) was 1.5 to 2 times faster in the R403Q myofibrils compared to controls. The isometric, linear, relaxation phase was shorter (ca 25%) and its rate constant (slow k_{REL}) was more than 3 times faster in the R403Q myofibrils than in both control groups, indicative of faster crossbridge detachment under isometric conditions.

Discussion

The results provide evidence that the apparent rate with which force-generating crossbridges detach under isometric conditions as well as the overall crossbridge

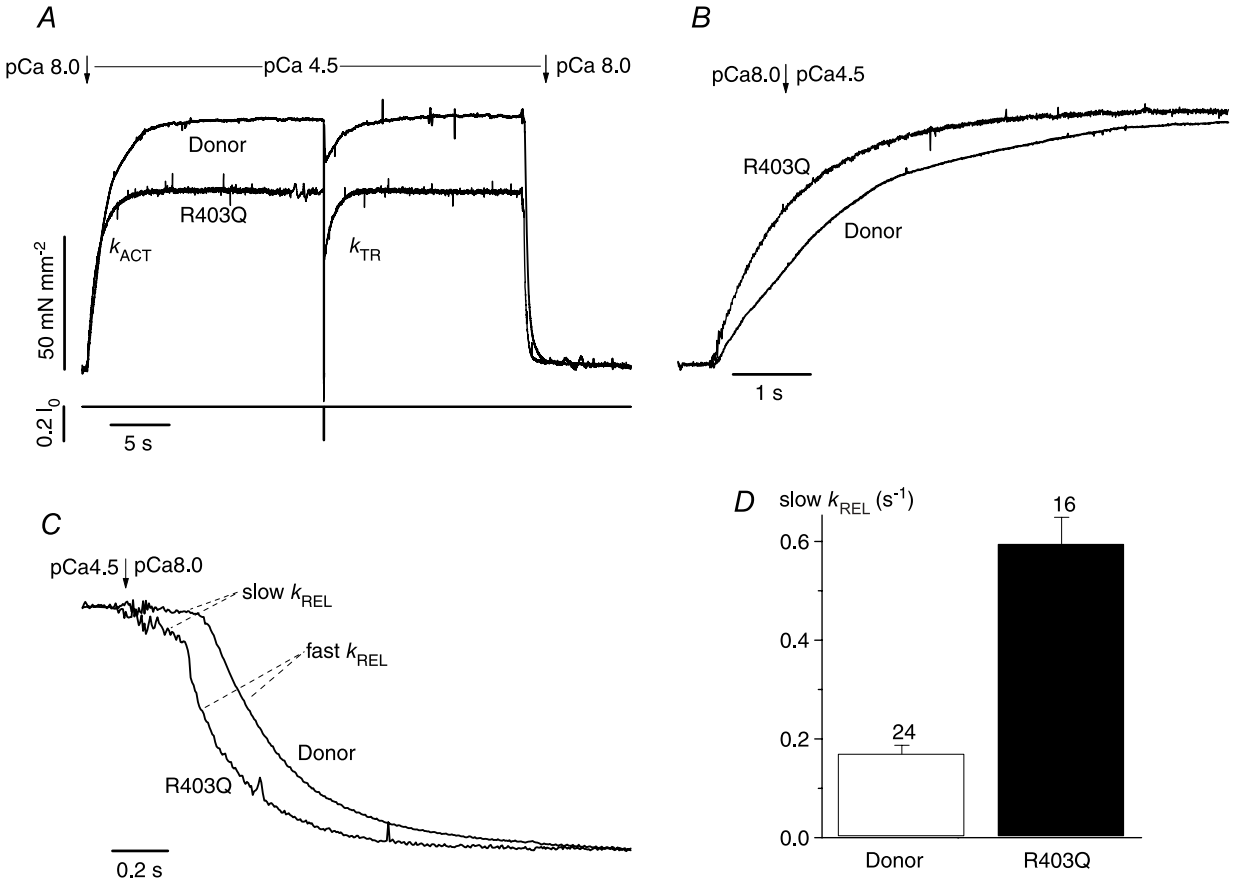

Figure 1. Tension generation and relaxation in control donor and R403Q heart myofibrils
A, representative tension responses (top traces) of donor and R403Q myofibrils maximally activated and fully relaxed by fast solution switching (pCa changes at arrows as indicated). Temperature 15°C. Fast length changes (bottom traces) are applied to the myofibrils under conditions of steady tension generation. k_{ACT} is the rate constant of tension generation following fast Ca^{2+} activation; k_{TR} is the rate constant of tension redevelopment following the release–restretch. *B*, the time course of tension activation following sudden [Ca^{2+}] increase of the donor and R403Q myofibrils shown in *A* are superimposed on a faster time base after normalization for maximal tension. *C*, tension relaxation kinetics following sudden Ca^{2+} removal; same traces as in *A* superimposed on a faster time base after normalization for maximal tension. The rate constant of the early slow force decline (slow k_{REL}) is estimated from the slope of the regression line fitted to the tension trace normalized to the entire amplitude of the tension relaxation transient. The rate constant for the final fast phase of tension decline (fast k_{REL}) is estimated from mono-exponential fit. *D*, mean values of slow k_{REL} for the donor (open columns) and R403Q (filled columns) myofibrils. Bars above columns are S.E.M.; the number of myofibrils is given above S.E.M. bars.

Table 1. Means (± S.E.M.) of resting parameters, maximum active tension (P_0), and kinetic data for full tension generation and relaxation in R403Q and control myofibrils

	R403Q	Control donor	Control patients[1]
Resting conditions			
Sarcomere length (μm)	2.23 ± 0.05 (13)	2.21 ± 0.02 (22)	2.19 ± 0.01 (62)
Passive tension (mN mm^{-2})	9.4 ± 1.9 (16)	9.9 ± 1.5 (19)	10.1 ± 1.1 (53)
Active tension generation			
P_0 (mN mm^{-2})	60 ± 7§ (16)	92 ± 12 (19)	108 ± 7 (53)
k_{ACT} (s^{-1})	1.40 ± 0.15*§ (18)	0.84 ± 0.06 (22)	0.73 ± 0.03 (62)
k_{TR} (s^{-1})	1.37 ± 0.16*§ (18)	0.77 ± 0.04 (22)	0.68 ± 0.03 (55)
Full tension relaxation			
Slow phase duration (ms)	169 ± 10*§ (16)	225 ± 10 (22)	226 ± 8 (47)
Slow k_{REL} (s^{-1})	0.60 ± 0.05*§ (16)	0.17 ± 0.02 (22)	0.15 ± 0.02 (47)
Fast k_{REL} (s^{-1})	5.12 ± 0.34*§ (16)	2.91 ± 0.16 (22)	2.90 ± 0.16 (47)

[1]Data from Piroddi *et al.* (2007), pooled from 4 patients. *$P < 0.01$ R403Q *versus* control donor, §$P < 0.01$ R403Q *versus* control patients (estimated by Student s *t* test; number of myofibrils in parentheses). Experimental conditions: 15°C; pCa of relaxing and activating solutions, 8.00 and 4.50, respectively; [MgATP] 5 mM; [P_i] < 5 μM.

turnover are markedly accelerated in the R403Q preparations. In the absence of inorganic phosphate (P_i), as in the present experiments, crossbridges leave their force-generating states through transitions that lead to ATP hydrolysis steps (Gordon *et al.* 2000; Poggesi *et al.* 2005) (Fig. 2*A*) and the faster crossbridge detachment rate associated with the R403Q mutation will generate higher energy costs to produce a given tension (higher

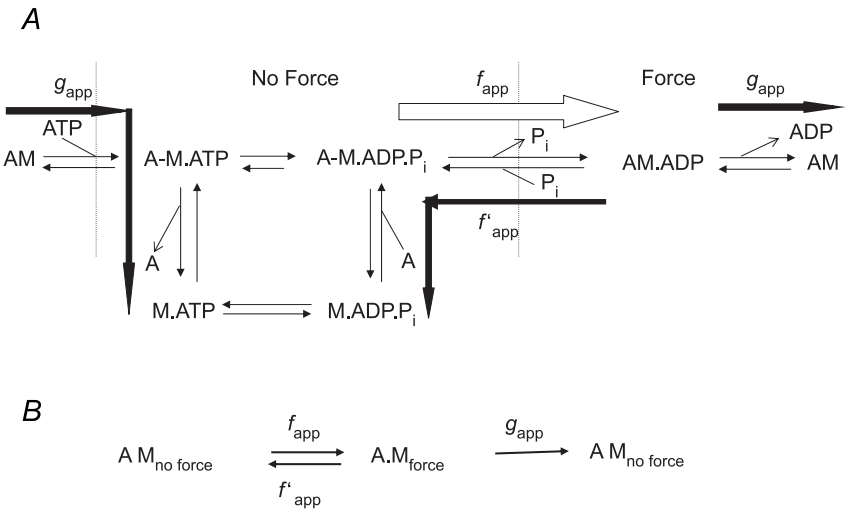

Figure 2. Reaction pathway for acto-myosin ATPase and energy transduction cycle
A, in this generally accepted scheme (Gordon *et al.* 2000), nucleotide occupancy of the myosin (M) active site modifies myosin s binding affinity for actin (A), defining two general categories of crossbridges: 'strong binding states' (AM, AM.ADP) and 'weak binding states' (AM.ATP, AM.ADP.P_i); crossbridges in strong states may generate force; crossbridges in weak states do not and are mostly detached forms (M.ATP, M.ATP.P_i). The release of inorganic phosphate (P_i) is thought to power the working stroke. It is well established that crossbridge transitions involved in P_i release and force generation are reversible and, in the presence of P_i, both forward (ADP release and ATP binding) and backward (P_i rebinding and reversal of the power stroke) transitions of crossbridges from force-generating to non-force-generating states occur. However, myofibril slow k_{REL}, measured in the absence of P_i, only probes forward detachment transitions that lead to ATP hydrolysis steps. *B*, 2-state crossbridge scheme. $AM_{no\ force}$ represents all weak binding states (mostly detached) and AM_{force} all strong binding states. The transition from the non-force-generating states to the force-generating states has an apparent rate constant f_{app} whereas g_{app} describes the return to the non-force-generating states by means of ADP release and ATP binding. The apparent rate constant for the reverse transition f'_{app} that depends on [P_i] can be neglected under the conditions of the present study.

ATPase/tension ratio) (de Tombe & Stienen, 2007). The increase in slow k_{REL} observed in R403Q sarcomeres implies that the increase in crossbridge detachment rates is the major factor responsible for the faster isometric crossbridge turnover (faster k_{ACT} and k_{TR}) and the lower maximal isometric tension. According to a simple 2-state model of acto-myosin interactions (Brenner, 1988) (Fig. 2*B*) in which the formation of force-generating crossbridges occurs via an apparent rate constant f_{app}, whereas crossbridge detachment occurs with apparent rate constant g_{app}, the overall cross-bridge turnover rate is given by $f_{app} + g_{app}$, tension is proportional to $f_{app}/(f_{app} + g_{app})$, and the energy cost of tension generation (ATPase/tension ratio) is proportional to g_{app} (Brenner, 1988; de Tombe & Stienen, 2007). Given slow $k_{REL} \approx g_{app}$ ($0.17 \pm 0.02\ s^{-1}$ and $0.60 \pm 0.05\ s^{-1}$ in control donor and R403Q myofibrils, respectively) and $k_{TR} \approx f_{app} + g_{app}$ ($0.77 \pm 0.04\ s^{-1}$ and $1.37 \pm 0.16\ s^{-1}$ in control donor and R403Q myofibrils, respectively), the increase in g_{app} associated with the R403Q mutation accounts almost entirely for the changes in P_0 and k_{TR} (as well as k_{ACT}) observed in these experiments and predicts a 3-fold increase in the energy cost of tension generation in R403Q sarcomeres. Though only direct measurements of ATPase activity can definitely confirm this conclusion, the results strongly support the idea that the R403Q mutation leads to inefficient ATP utilization for tension generation.

Inefficient ATP utilization for tension generation may lead to an energetic defect that results in cardio-myocyte dysfunction and hypertrophy (Crilley *et al.* 2003; Ashrafian *et al.* 2003). If myocardial wall tension and energy demand are not uniformly distributed in the left ventricle as suggested by theoretical models (DeAnda *et al.* 1998) and experimental studies (Dunn, 1984), extra energy requirements could be more damaging in specific myocardial regions providing an explanation for the asymmetrical hypertrophy and degenerative changes observed in the interventricular septum of the patient (see Supplemental file).

The present experiments focus on the functional properties of cardiac myofilaments independent of disease-associated secondary changes occurring in other cell functions and sarcomere ionic microenvironment. It is more certain therefore that the faster kinetics observed in the R403Q myofibrils are due to the functional properties of the mutant protein. Kinetic parameters of the R403Q myofibrils were the fastest human ventricular parameters ever measured in our laboratory. In contrast, tension activation and relaxation kinetics were slower or tended to be slower in myofibrils from secondary hypertrophic and failing human hearts (authors unpublished results), evidence that present results do not reflect myofilament changes secondary to the disease (myofilament Ca^{2+} sensitivity as well as sarcomeric protein composition of the R403Q myofibrils are under investigation on leftover tissue of the patient sample). Whereas initial functional studies had suggested that the R403Q mutation depresses acto-myosin interaction kinetics and contractile function potentially leading to a hypertrophic compensatory response (Cuda *et al.* 1993; Lankford *et al.* 1995; Sata & Ikebe, 1996), more recent work seemed to imply that the mutant myosin has enhanced motor activity (Tyska *et al.* 2000; Lowey, 2002; Keller *et al.* 2004). Interestingly, pure mutant myosin isolated from the heart of a patient with homozygous R403W mutation showed a 2-fold increase in actin-activated ATPase activity and the K_m for actin indicating both a large enhancement of the enzymatic properties and a lower affinity for actin (Keller *et al.* 2004). Our mechanical observations agree well with these biochemical findings.

Our finding that relaxation kinetics are faster in the R403Q myofibrils than in the controls is apparently inconsistent with clinical evidence of diastolic dysfunction associated with FHC (Ho *et al.* 2002). In the whole heart, however, multiple mechanisms determine the time course of tension relaxation. For example, the extensive fibrosis found at histological examination of the myectomy samples (see Supplemental Fig. 1*D*) may contribute to the clinically observed impairment in ventricular relaxation. Moreover, in intact cardiac myocytes the mechanisms related to myoplasmic $[Ca^{2+}]$ reduction are important determinants of the time course of tension relaxation. With fast solution switching on myofibrils we focus solely on the sarcomeric determinants of the relaxation process and, under our present experimental conditions (absence of P_i, Fig. 2), we only probe one specific pathway through which crossbridges leave force-generating states (Gordon *et al.* 2000; Poggesi *et al.* 2005). In the intact myocardium, compromised energetics may slow down relaxation by altering the ionic environment of contractile proteins and/or the Ca^{2+} transport function needed for myo-plasmic $[Ca^{2+}]$ to fall. Elevated myoplasmic $[Ca^{2+}]$ and altered function of other ion transporters needed for normal electrophysiological activity may also render the myocardium vulnerable to the arrhythmias that underlie sudden cardiac death in FHC.

Present results on human cardiac myofibrils add substantial support to the hypothesis that inefficient utilization of ATP, hence compromised energetics, plays a central role in HCM. ^{31}P NMR spectroscopy studies in transgenic mouse (Spindler *et al.* 1998; Javadpour *et al.* 2003) and human hearts (Crilley *et al.* 2003) bearing FHC-associated mutations in sarcomeric proteins found a large fall in phosphocreatine concentration ([PCr]) but unchanged ATP levels. The [PCr] fall was also present in R403Q transgenic mouse hearts without hypertrophy (Spindler *et al.* 1998) and in asymptomatic FHC patients (Crilley *et al.* 2003), implying that the energetic defect was associated with the mutant sarcomeric proteins, not to left ventricular hypertrophy per se. Finally, FHC-like

phenotypes have been found with mutations in a variety of metabolic genes and in AMP-dependent protein kinase, an enzyme regulating substrate selection for ATP synthesis in muscle (Ashrafian *et al.* 2003; Crilley *et al.* 2003). These provide further support for defective energetics as being pivotal in understanding FHC disease.

References

Ashrafian H, Redwood C, Blair E & Watkins H (2003). Hypertrophic cardiomyopathy: a paradigm for myocardial energy depletion. *Trends Genet* **19**, 263–268.

Belus A, Piroddi N, Scellini B, Tesi C, Olivotto I, Cecchi F & Poggesi C (2007). Kinetics of force generation and relaxation in human cardiac myofibrils from FHC patient carrying the R403Q mutation in myosin heavy chain. *Biophys J* **92** (Suppl.), 181a.

Brenner B (1988). Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibres: implications for regulation of muscle contraction. *Proc Natl Acad Sci U S A* **85**, 3265–3269.

Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K & Watkins H (2003). Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol* **41**, 1776–1782.

Cuda G, Fananapazir L, Zhu WS, Sellers JR & Epstein ND (1993). Skeletal muscle expression and abnormal function of beta-myosin in hypertrophic cardiomyopathy. *J Clin Invest* **91**, 2861–2865.

DeAnda A Jr, Komeda M, Moon MR, Green GR, Bolger AF, Nikolic SD, Daughters GT 2nd & Miller DC (1998). Estimation of regional left ventricular wall stresses in intact canine hearts. *Am J Physiol Heart Circ Physiol* **275**, H1879–H1885.

de Tombe PP & Stienen GJ (2007). Impact of temperature on cross-bridge cycling kinetics in rat myocardium. *J Physiol* **584**, 591–600.

Dunn RB (1984). High energy phosphate depletion and lactate accumulation in the interventricular septum and left ventricular free wall of the dog after total coronary occlusion. *Circ Res* **54**, 405–413.

Geisterfer-Lowrance AA, Christe M, Conner DA, Ingwall JS, Schoen FJ, Seidman CE & Seidman JG (1996). A mouse model of familial hypertrophic cardiomyopathy. *Science* **272**, 731–734.

Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg HP, McKenna W, Seidman CE & Seidman JG (1990). A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell* **62**, 999–1006.

Gordon AM, Homsher E & Regnier M (2000). Regulation of contraction in striated muscle. *Physiol Rev* **80**, 853–924.

Ho C, Sweitzer NK, McDonough B, Maron BJ, Casey SA, Seidman JG, Seidman CE & Solomon SD (2002). Assessment of diastolic function with Doppler tissue imaging to predict genotype in preclinical hypertrophic cardiomyopathy. *Circulation* **105**, 2992–2997.

Javadpour MM, Tardiff JC, Pinz I & Ingwall JS (2003). Decreased energetics in murine hearts bearing the R92Q mutation in cardiac troponin T. *J Clin Invest* **112**, 768–775.

Keller DI, Coirault C, Rau T, Cheav T, Weyand M, Amann K, Lecarpentier Y, Richard P, Eschenhagen T & Carrier L (2004). Human homozygous R403W mutant cardiac myosin presents disproportionate enhancement of mechanical and enzymatic properties. *J Mol Cell Cardiol* **36**, 355–362.

Lankford EB, Epstein ND, Fananapazir L & Sweeney HL (1995). Abnormal contractile properties of muscle fibers expressing beta-myosin heavy chain gene mutations in patients with hypertrophic cardiomyopathy. *J Clin Invest* **95**, 1409–1414.

Lowey S (2002). Functional consequences of mutations in the myosin heavy chain at sites implicated in familial hypertrophic cardiomyopathy. *Trends Cardiovasc Med* **12**, 348–354.

Marian AJ, Wu Y, Lim DS, McCluggage M, Youker K, Yu QT, Brugada R, DeMayo F, Quinones M & Roberts R (1999). A transgenic rabbit model for human hypertrophic cardiomyopathy. *J Clin Invest* **104**, 1683–1692.

Piroddi N, Belus A, Scellini B, Tesi C, Giunti G, Cerbai E, Mugelli A & Poggesi C (2007). Tension generation and relaxation in single myofibrils from human atrial and ventricular myocardium. *Pflugers Arch* **454**, 63–73.

Poggesi C, Tesi C & Stehle R (2005). Sarcomeric determinants of striated muscle relaxation kinetics. *Pflugers Arch* **449**, 505–517.

Sata M & Ikebe M (1996). Functional analysis of the mutations in the human cardiac beta-myosin that are responsible for familial hypertrophic cardiomyopathy. Implication for the clinical outcome. *J Clin Invest* **98**, 2866–2873.

Spindler M, Saupe KW, Christe ME, Sweeney HL, Seidman CE, Seidman JG & Ingwall JS (1998). Diastolic dysfunction and altered energetics in the alphaMHC403/+ mouse model of familial hypertrophic cardiomyopathy. *J Clin Invest* **101**, 1775–1783.

Tesi C, Piroddi N, Colomo F & Poggesi C (2002). Relaxation kinetics following sudden Ca^{2+} reduction in single myofibrils from skeletal muscle. *Biophys J* **83**, 2142–2151.

Tyska MJ, Hayes E, Giewat M, Seidman CE, Seidman JG & Warshaw DM (2000). Single molecule mechanics of R403Q cardiac myosin isolated from the mouse model of familial hypertrophic cardiomyopathy. *Circ Res* **86**, 737–744.

Acknowledgements

Major financial support by Telethon-Italy (grant no. GGP07133) is gratefully acknowledged. This work was also partially supported by the Ministery of University and Research (COFIN 2006) and by Ente Cassa di Risparmio di Firenze.

Supplemental material

Online supplemental material for this paper can be accessed at:
http://jp.physoc.org/cgi/content/full/jphysiol.2008.155952/DC1
and
http://www.blackwell-synergy.com/doi/suppl/10.1113/jphysiol.2008.155952