Quantification of the Effects of Electrical Remodeling due to Hypertrophic Cardiomyopathy on Human Ventricular Electromechanical Activity and Energetics

Gareth M Jones, Michael A Colman, Henggui Zhang

Biological Physics Group, University of Manchester, Manchester, UK

Abstract

Hypertrophic cardiomyopathy caused by mutations in genes coding for sarcomeric proteins leads to electrical remodeling, oxidative stress and energetic impairment. This study combines recently published models of each component to investigate the mechanisms by which the hypertrophic cardiomyopathy-induced electrical remodeling, oxidative stress and energetic malfunction affect cardiac electrical activities.

A current electrophysiological model was updated with recent experimental data for both control and disease. A metabolite sensitive myofilament model was incorporated alongside a model of mitochondrial energetics and reactive oxygen species production. It was shown that electrical remodeling leads to a substantial prolongation of action potential duration, an increase in both systolic and diastolic Ca^{2+} levels and concomitant increase in levels of active Ca^{2+}/calmodulin kinase II. A positive inotropic effect was seen with hypertrophic cardiomyopathy due to increased Ca^{2+} concentration and Ca^{2+} sensitivity of the myofilament. Greater mitochondrial reactive oxygen species production was observed in the diseased cell and energetic buffering capacity by phosphocreatine was found to be diminished.

1. Introduction

Hypertrophic cardiomyopathy (HCM) is an inherited autosomal dominant disease affecting 0.02% of the population worldwide [1]. Primarily a disease of the sarcomere it can lead to severe irregular hypertrophy of the left ventricle and sudden cardiac death. It is associated with energetic impairment due to inefficiency of mutant sarcomeric proteins and an increase in the calcium sensitivity of the myofilament. These factors lead to an increased Ca^{2+} load and impairment of cytosolic ATPases such as the sarcoplasmic reticulum $Ca2^{+}$-ATPase (SERCA), an increase in active Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and greater phosphorylation of its targets, such as the L-type Ca^{2+} channel, the ryanodine receptors (RyRs) and phospholamban [2]. Due to greater energetic consumption by the cardiac myofilament, mitochondria are placed under strain leading to energetic impairment and an increase in reactive oxygen species (ROS) production.

The cell's ability to respond quickly to increased workload is supplied by the phosphocreatine kinase buffer which allows for generation of ATP from ADP and phosphocreatine [1]. The ratio of phosphocreatine to ATP is used as a measure of energetic impairment and is found to be diminished with HCM [1].

Within healthy cells the concentration of ROS is constrained by cellular antioxidant defenses. These defenses reside within the mitochondrial matrix and normally control ROS levels to inhibit oxidative damage to the cell. Crucial to these defenses is nicotinamide adenine dinucleotide phosphate (NADPH) which acts to replenish the antioxidant ability of these defenses. A decrease in levels of NADPH may compromise the ability of the cell to regulate levels of ROS [3]. A reduction in antioxidant capacity may lead to the transport of ROS into the cytosol, leading to oxidative damage to other parts of the cell. CaMKII may also be activated by ROS resulting in deleterious effects and further electrical remodeling [4].

A recent study by Coppini *et al.* [2] identified the nature of the electrical remodeling seen with the disease. They found HCM prolonged the action potential duration, augmented the Ca^{2+} transient (CaT) and increased levels of active CaMKII. Data on myofilament Ca^{2+} sensitivity was also presented showing a left-shifted force calcium curve. In this study we developed a model incorporating this data to investigate how this electrical remodeling affects the contractile properties and the energetics of the cell.

2. Methods

The O'Hara-Rudy ventricular cell model [5] was coupled with a metabolite sensitive formulation for mechanical contraction by Tran *et al.* [6]. The energetics component was based on a model by Gauthier *et al.* of mitochondrial energetics, incorporating expressions for the electron transport chain and ROS production [7].

The O'Hara-Rudy model was updated by fitting I-V relationships of I_{CaL}, I_{to} and I_{K1} for both control and HCM

data provided by Coppini *et al*. (Figure 1). Other currents were scaled as per data provided by Coppini *et al.* Cell volume was increased by 90% and membrane capacitance by 59% to reflect the changes observed in hypertrophied cells.

To simulate increased activation and autophosphorylation of CaMKII due to prolonged Ca^{2+} overload the amount of active CaMKII within the cytosol was increased in line with data from Coppini *et al.* who observed that levels of autophosphorylated CaMKII were 3.5 times higher than in control cells.

The Tran *et al.* model was modified to simulate the experimentally observed increase in calcium sensitivity (Figure 2). These modifications were made by decreasing the Hill coefficient in the model along with the half activation calcium concentration. For force production measurements the initial sarcomere length was set to 2.2 um.

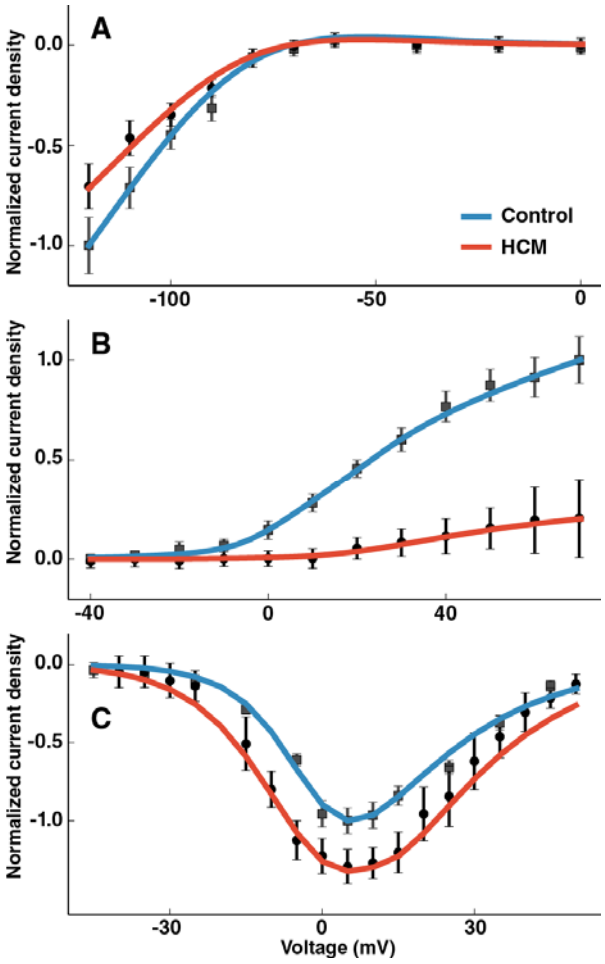

Figure 1. Experimental and simulated I-V relationships for **A**) I_{K1}, **B**) I_{to} and **C**) I_{CaL}. Squares and circles represent control and HCM data respectively. Both experimental and simulation data is normalized to the maximum of the control current [5].

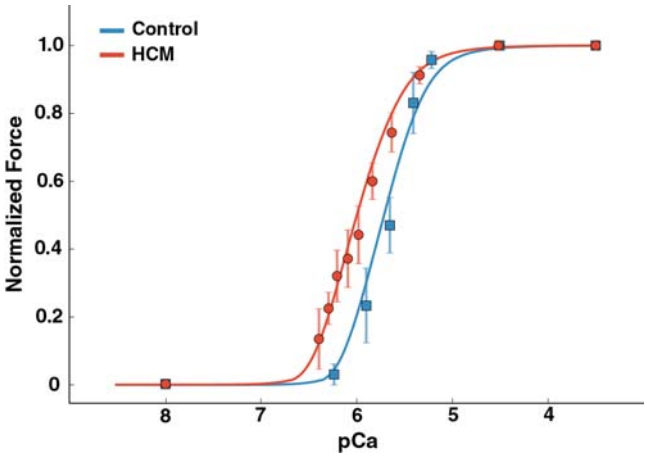

Figure 2. Force-calcium curve showing leftward shift of calcium sensitivity with disease. Blue squares and red circles denote experimental data of control and HCM cells respectively. pCa = -log10($[Ca^{2+}]_i$). Force was normalized to maximum for each cell.

Electrophysiological and contractile components of the cell were coupled with the energetics and ROS production model through intracellular Ca^{2+} concentration and metabolite levels. Expressions for cytosolic ATP and creatine kinase levels were incorporated from work by Cortassa *et al* [8], these include separate pools for use by cytosolic ATPases and the myofilament. The expressions for SERCA and the plasma membrane Ca^{2+}-ATPase were modified to include their uptake of ATP. To investigate the effect of CaMKII on ROS production levels of CaMKII were increased within physiological limits and its effect on ROS levels assessed.

3. Results

3.1. Effects of electrical remodeling

The model accurately reproduced experimentally observed changes in action potential duration at 90% of repolarization (APD_{90}) and the CaT. An increase in active force production by the myofilament model was observed in the HCM model compared to control. This was due to an increased calcium sensitivity and peak CaT. The model also reproduces the experimentally observed force-calcium relationship as seen in Figure 2.

Electrical remodeling due to HCM resulted in a 46% increase in APD_{90}, augmentation of the late sodium current was the main contributing factor to this change (Figure 3A). An increase in I_{CaL} amplitude led to an increased peak systolic Ca^{2+} concentration and decreased SERCA uptake to slower decay kinetics (Figure 3B). Increased cytosolic Ca^{2+} resulted in an 18% increase in levels of active CaMKII.

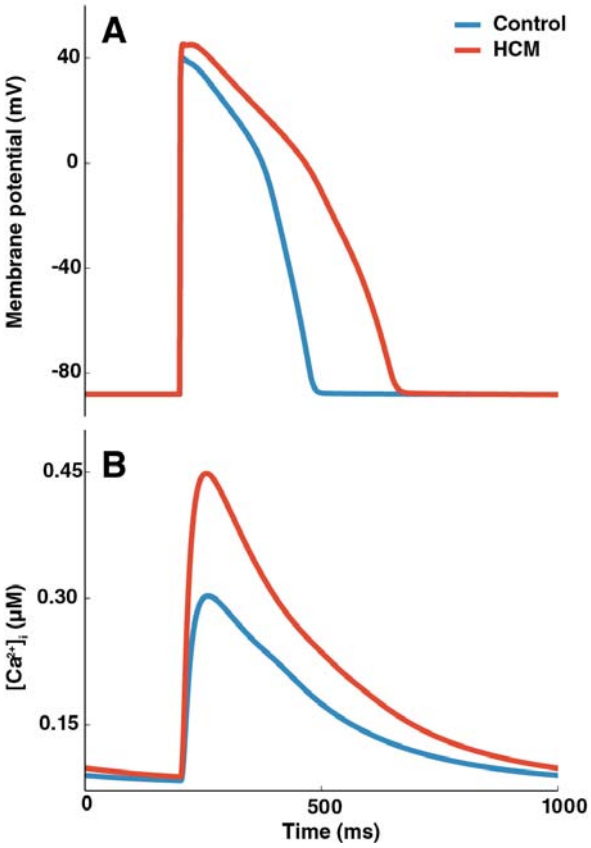

Figure 3. A) Changes in APD_{90} and AP morphology with disease. B) CaT showing greatly augmented peak C^{a2+} concentration and slower recovery. Simulation with a cycle length of 1000 ms.

3.2. Modified contractile properties with HCM

A leftward shift in the force-calcium relationship due to HCM resulted in a myofilament model with an increased calcium sensitivity. This led to a 46% increase in maximal force production (Figure 4A) and greater cell length shortening. These results are consistent with experimental findings of augmented force seen with HCM [1].

3.3. Energetic impairment and ROS

A higher level of energy consumption by the myofilament resulted in a 14% reduction of the PCr/ATP ratio inferring decreased buffering capacity to the cell. Ca^{2+} concentration within the cytosol was increased resulting in higher uptake into the mitochondrial matrix by the mitochondrial calcium uniporter, this in turn resulted in a stimulation of ATP production. Although generation of ATP was augmented through stimulation by Ca^{2+}, the increase did not compensate for the increased workload. With disease a 95% increase in ROS production was seen (Figure 4B). This was accompanied by a 26% decrease in mitochondrial NADPH levels which may show a compromised antioxidant capacity.

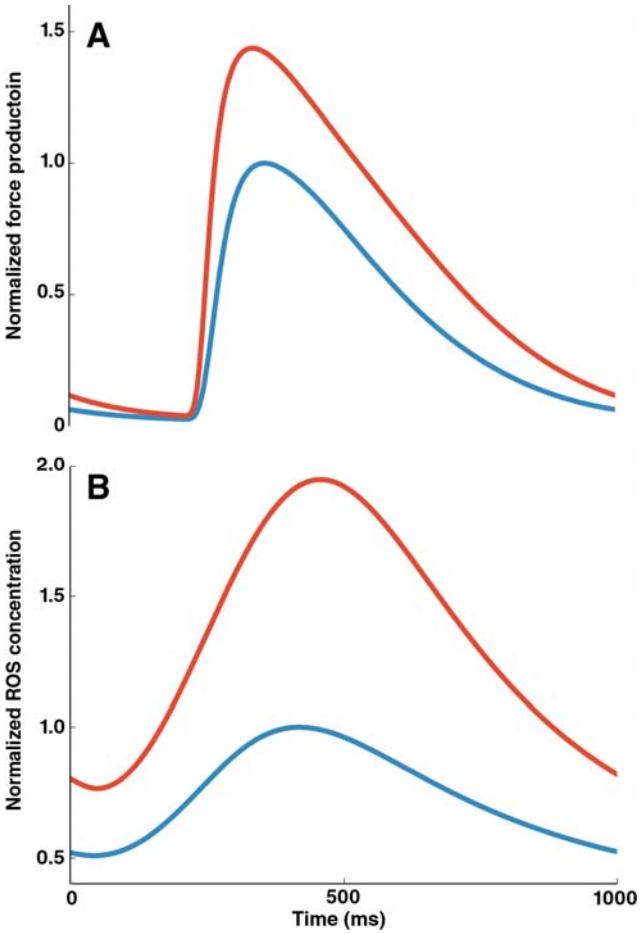

Figure 4. A) A large increase in force generation is seen with HCM. A slightly higher diastolic CaT results in incomplete relaxation of the myofilament before the next beat is initiated. B) Significant increase in ROS production with disease during both diastole and systole.

3.4. The effect of CaMKII levels on ROS production

Coppini *et al.* observed greatly increased levels of active CaMKII in HCM cells. Targets of CaMKII in the O'Hara-Rudy model include I_{CaL} SERCA and the RyRs. An increase in phosphorylated channels increases current amplitude resulting in an augmentation of cytosolic Ca^{2+} levels. With maximal scaling of active levels of CaMKII within the cytosol a 2.5 fold increase in mitochondrial ROS was seen (Figure 5).

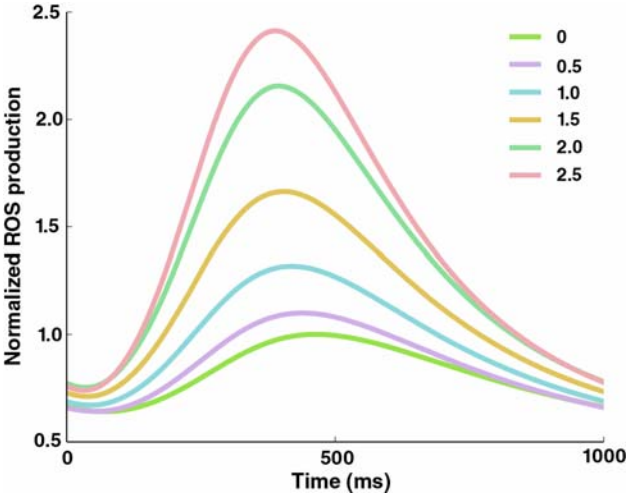

Figure 5. Increase in ROS production with scaling of active CaMKII levels. Values in the legend represent scaling factor of active CaMKII concentration. Levels were scaled within physiological values and normalized to maximum value at zero active CaMKII.

4. Discussion

In this study a model reproducing recent experimental data for both control and HCM cardiomyocytes was developed. This model allows for investigation into how changes in one aspect of a cell lead may to deleterious changes in another. Our results show the effects increased Ca^{2+} sensitivity and electrical remodeling have on the contractile properties of the cell, energetics, and levels of ROS production. Electrical remodeling leads to prolongation of APD_{90}, a reduction in repolarizing potassium currents and an increase in cytosolic Ca^{2+} concentration. Activity of CaMKII was found to be higher in HCM cells reflecting changes observed experimentally [2].

Increased myofilament Ca^{2+} sensitivity led to a larger contractile force and greater cell length shortening in HCM myocytes. Changes in myofilament properties also led to an increase in ATP utilization resulting in energetic compromise within the cell. This was evident in buffering capacity impairment, seen through a reduction in the PCr/ATP ratio, magnitude of this reduction closely matched experimental results [9].

ROS production augmentation was evident in the HCM myocyte due to increased workload and greater CaMKII activation. Increasing levels of CaMKII led to greater ROS production. Modelling ROS activation of CaMKII is a topic of interest for further study.

Acknowledgments

This work was supported by a project grant from EPSRC UK (EP/J00958X/1)

References

[1] Vakrou S, Abraham MR. Hypertrophic cardiomyopathy: a heart in need of an energy bar? Front. Physiol. 2014;5:309

[2] Coppini R, Ferrantini C, Yao L, Fan P, Del Lungo M, Stillitano F, et al. Late sodium current inhibition reverses electromechanical dysfunction in human hypertrophic cardiomyopathy. Circulation. 2013;127:575–84.

[3] Murphy MP. How mitochondria produce reactive oxygen species. Biochem. J. 2009;417:1–13.

[4] Luczak ED, Anderson ME. CaMKII oxidative activation and the pathogenesis of cardiac disease. J. Mol. Cell. Cardiol 2014;73:112–6.

[5] O'Hara T, Virág L, Varró A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. PLoS Comput. Biol. 2011;7:e1002061.

[6] Tran K, Smith NP, Loiselle DS, Crampin EJ. A metabolite-sensitive, thermodynamically constrained model of cardiac cross-bridge cycling: Implications for force development during ischemia. Biophys. J. Biophysical Society; 2010;98:267–76.

[7] Gauthier LD, Greenstein JL, O'Rourke B, Winslow RL. An integrated mitochondrial ROS production and scavenging model: Implications for heart failure. Biophys. J. Biophysical Society 2013;105:2832–42.

[8] Cortassa S, Aon M a, O'Rourke B, Jacques R, Tseng H-J, Marbán E, et al. A computational model integrating electrophysiology, contraction, and mitochondrial bioenergetics in the ventricular myocyte. Biophys. J. 2006;91:1564–89.

[9] Esposito a, De Cobelli F, Perseghin G, Pieroni M, Belloni E, Mellone R, et al. Impaired left ventricular energy metabolism in patients with hypertrophic cardiomyopathy is related to the extension of fibrosis at delayed gadolinium-enhanced magnetic resonance imaging. Heart. 2009;95:228–33.

Address for correspondence.

Gareth Jones
Biological Physics Group
Schuster Building,
The University of Manchester,
Manchester,
M13 9PL
UK
gareth.jones-15@postgrad.manchester.ac.uk