Why Is there a Limit to the Changes in Myofilament Ca$^{2+}$-Sensitivity Associated with Myopathy Causing Mutations?

Steven B. Marston*

National Heart & Lung Institute, Imperial College London, London, UK

Mutations in striated muscle contractile proteins have been found to be the cause of a number of inherited muscle diseases; in most cases the mechanism proposed for causing the disease is derangement of the thin filament-based Ca$^{2+}$-regulatory system of the muscle. When considering the results of experiments reported over the last 15 years, one feature has been frequently noted, but rarely discussed: the magnitude of changes in myofilament Ca$^{2+}$-sensitivity due to myopathy-causing mutations in skeletal or heart muscle seems to be always in the range 1.5–3x EC$_{50}$. Such consistency suggests it may be related to a fundamental property of muscle regulation; in this article we will investigate whether this observation is true and consider why this should be so. A literature search found 71 independent measurements of HCM mutation-induced change of EC$_{50}$ ranging from 1.15 to 3.8-fold with a mean of 1.87 ± 0.07 (sem). We also found 11 independent measurements of increased Ca$^{2+}$-sensitivity due to mutations in skeletal muscle proteins ranging from 1.19 to 2.7-fold with a mean of 2.00 ± 0.16. Investigation of dilated cardiomyopathy-related mutations found 42 independent determinations with a range of EC$_{50}$ wt/mutant from 0.3 to 2.3. In addition we found 14 measurements of Ca$^{2+}$-sensitivity changes due skeletal muscle myopathy mutations ranging from 0.39 to 0.63. Thus, our extensive literature search, although not necessarily complete, found that, indeed, the changes in myofilament Ca$^{2+}$-sensitivity due to disease-causing mutations have a bimodal distribution and that the overall changes in Ca$^{2+}$-sensitivity are quite small and do not extend beyond a three-fold increase or decrease in Ca$^{2+}$-sensitivity. We discuss two mechanism that are not necessarily mutually exclusive. Firstly, it could be that the limit is set by the capabilities of the excitation-contraction machinery that supplies activating Ca$^{2+}$ and that striated muscle cannot work in a way compatible with life outside these limits; or it may be due to a fundamental property of the troponin system and the permitted conformational transitions compatible with efficient regulation.

Keywords: muscle regulation, Ca$^{2+}$-sensitivity, troponin C, HCM, DCM, myopathy, mutation

Abbreviations: HCM, hypertrophic cardiomyopathy; RCM, Restrictive cardiomyopathy; DCM, dilated cardiomyopathy; EC$_{50}$, Ca$^{2+}$ concentration that gives 50% maximal activation; pCa$_{50}$, –log EC$_{50}$.
Mutations in striated muscle contractile proteins have been found to be the cause of a number of inherited muscle diseases; in most cases the mechanism proposed for causing the disease is derangement of the thin filament-based Ca$^{2+}$-regulatory system of the muscle. Hypertrophic cardiomyopathy and hypercontractile diseases of skeletal muscle, such as distal arthrogryposis and “stiff child syndrome,” have been linked to a higher myofilament Ca$^{2+}$-sensitivity (Marston, 2011; Donkervoort et al., 2015). In contrast dilated cardiomyopathy mutations are commonly, but not exclusively, linked to decreased Ca$^{2+}$-sensitivity. Mutations in contractile proteins that are linked to nemaline myopathy and related skeletal muscle myopathies have also been found to be associated with reduced Ca$^{2+}$-sensitivity (Martilha et al., 2012, 2014). The causative connection between myofilament Ca$^{2+}$-sensitivity and muscle dysfunction is a field of intensive research that is too complex to consider in this account. However, when considering the results of such experiments reported over the last 15 years, one feature has been frequently noted, but rarely discussed. The magnitude of changes in myofilament Ca$^{2+}$-sensitivity due to myopathy-causing mutations in skeletal or heart muscle seems to be always in the range 1.5–3x EC$_{50}$. Such consistency suggests it may be related to a fundamental property of muscle regulation; in this article we will investigate whether this observation is true and consider why this should be so.

Most investigations have found increased Ca$^{2+}$-sensitivity in muscle with hypertrophic cardiomyopathy (HCM) and restrictive cardiomyopathy (RCM)-causing mutations. Our literature search found 71 independent measurements of the mutation-induced change of EC$_{50}$ ranging from 1.15 to 3.8-fold with a mean of 1.87 ± 0.07 (sem) (Table 1). We also found 11 independent measurements of increased Ca$^{2+}$-sensitivity due to mutations in skeletal muscle proteins ranging from 1.19 to 2.7-fold with a mean of 2.00 ± 0.16 (Table 2).

Dilated cardiomyopathy-causing mutations were initially found to decrease Ca$^{2+}$-sensitivity but more recent studies have indicated the situation is more complex. DCM-linked mutations can both increase and decrease Ca$^{2+}$-sensitivity depending on the individual mutations, moreover the direction of change can be different with a single mutation measured in different systems (Marston, 2011; Memo et al., 2013). This is illustrated in Table 3 where 42 independent determinations show a range of EC$_{50}$ wt/mutant from 0.3 to 2.3. In addition we found 14 measurements of Ca$^{2+}$-sensitivity changes due skeletal muscle myopathy mutations ranging from 0.39 to 0.63 (Table 4).

Thus, our extensive literature search, although not necessarily complete, found that, indeed, the changes in myofilament Ca$^{2+}$-sensitivity due to disease-causing mutations have a bimodal distribution and that the overall changes in Ca$^{2+}$-sensitivity are quite small and do not extend beyond a 3–4-fold increase or decrease in Ca$^{2+}$-sensitivity. Indeed when all the findings are plotted as a histogram one finds that increases in Ca$^{2+}$-sensitivity on a log scale have an approximately normal distribution with mean increase in Ca$^{2+}$-sensitivity (EC$_{50}$ wt/mutant) of 1.86-fold (corresponding to $\Delta$EC$_{50}$ = 0.255 ± 0.015), whilst the decreases in Ca$^{2+}$-sensitivity have a mean EC$_{50}$ wt/mutant of 0.54-fold (corresponding to $\Delta$EC$_{50}$ of $-0.286 \pm 0.01$; Figure 1A). It

| Gene name | Mutation | wt/mutant EC$_{50}$ ratio | Measured in | References |
|-----------|----------|--------------------------|-------------|------------|
| ACTC      | E99K     | 2.45                     | NMA         | Song et al., 2011 |
| ACTC      | E99K     | 1.24                     | NMA (human) | Song et al., 2011 |
| ACTC      | E99K     | 1.89                     | NMA         | Papadakis et al., 2015 |
| ACTC      | E99K     | 1.3                      | Fibers TG   | Song et al., 2011 |
| ACTC      | E99K     | 2.35                     | Myofilbrils TG | Song et al., 2013 |
| MYL2      | R58Q     | 1.29                     | Fibers X    | Szczesna-Cordary et al., 2004 |
| MYL2      | D166V    | 1.78                     | Fibers TG   | Kerrick et al., 2009 |
| MYL2      | D166V    | 1.82                     | Fibers TG   | Yuan et al., 2015 |
| MYH7      | R403Q    | 1.79                     | Human fibers | Sequera et al., 2013 |
| MYH7      | R403Q    | 1.41                     | Fibers TG   | Blanchard et al., 1999 |
| MYH7      | R453Q    | 1.99                     | Human fibers | Palmer et al., 2004 |
| MYBP3     | Cat R820W| 2.01                     | NMA         | Messer et al., 2016a |
| MYBP3     | "K"     | 1.35                     | Fibers TG   | Frayse et al., 2012 |
| MYBP3     | E258K    | 1.80                     | Human fibers | Sequera et al., 2013 |
| TNNC1     | A8V      | 2.51                     | Fibers TG   | Martins et al., 2015 |
| TNNC1     | A8V      | 2.3                      | Fibers X    | Pinto et al., 2009 |
| TNNC1     | L29Q     | 1.26                     | Fibers X 2.3 µm | Li et al., 2013 |
| TNNC1     | L29Q     | 1.17                     | Fibers X 1.9 µm | Li et al., 2013 |
| TNNC1     | L29Q     | 2.1                      | NMA         | Schmidtmann et al., 2005 |
| TNNC1     | A31S     | 1.48                     | Fibers X    | Parvatiyar et al., 2012 |
| TNNC1     | A31S     | 2.75                     | ATPase      | Parvatiyar et al., 2012 |
| TNNC1     | D145E    | 1.74                     | Fibers X    | Pinto et al., 2009 |
| TNNC1     | C84Y     | 1.86                     | Fibers X    | Pinto et al., 2009 |
| TNN3      | R21C     | 2.16                     | Fibers X    | Gomes et al., 2005a |
| TNN3      | L144Q    | 2.04                     | Fibers X    | Gomes et al., 2005b |
| TNN3      | R145Q    | 3.63                     | ATPase      | Elliott et al., 2000 |
| TNN3      | R145Q    | 2.09                     | ATPase      | Takahashi-Yanaga et al., 2001 |
| TNN3      | R145Q    | 1.82                     | NMA         | Brunet et al., 2014 |
| TNN3      | R145Q    | 1.41                     | NMA         | Deng et al., 2001 |
| TNN3      | R145Q    | 1.35                     | Fibers X    | Lang et al., 2002 |
| TNN3      | R145Q    | 1.15                     | Fibers X    | Krüger et al., 2005 |
| TNN3      | R145Q    | 1.41                     | Fibers X    | Takahashi-Yanaga et al., 2001 |
| TNN3      | R145Q    | 1.70                     | ATPase      | Takahashi-Yanaga et al., 2001 |
| TNN3      | R145Q    | 2.45                     | Fibers X    | Gomes et al., 2005b |
| TNN3      | R145W    | 1.15                     | Human fibers | Sequera et al., 2013 |
| TNN3      | R162W    | 1.28                     | ATPase      | Takahashi-Yanaga et al., 2001 |
| TNN3      | A171I    | 1.38                     | Fibers X    | Gomes et al., 2005b |
| TNN3      | K178E    | 2.95                     | Fibers X    | Gomes et al., 2005b |
| TNN3      | ΔK182    | 1.51                     | ATPase      | Takahashi-Yanaga et al., 2001 |
| TNN3      | ΔK183    | 3.8                      | NMA         | Köhler et al., 2003 |
| TNN3      | R192H    | 2.29                     | Fibers X    | Gomes et al., 2005b |
| TNN3      | G203S    | 3.02                     | NMA         | Köhler et al., 2003 |
is also worth noting that this small Ca\textsuperscript{2+}-sensitivity shift is observed independent of the measurement method. Figure 1B compares the ΔCa\textsubscript{350} distribution measured by unloaded assays (actomyosin ATPase or in vitro motility) and by loaded assays (force measurements in skinned muscles, cell, and isolated myofibrils). The mean magnitude of the Ca\textsuperscript{2+}-sensitivity change is about 20% less when measured in loaded assays.

What could be the underlying reason for this consistent and small effect of mutations on EC\textsubscript{50}? We will consider two possible mechanisms that are not necessarily mutually exclusive. Firstly, it could be that the limit is set by the capacity of the EC coupling system that supplies activating Ca\textsuperscript{2+} and that striated muscle cannot work in a way compatible with life outside these limits; alternatively it may be due to a fundamental property of the troponin system and the permitted conformational transitions compatible with efficient regulation.

Before attempting to discuss these mechanisms it is worthwhile considering some additional evidence on Ca\textsuperscript{2+}-sensitivity shifts. Perhaps the most puzzling observation is that the physiological modulation of cardiac sensitivity shift and disease severity. Skeletal myopathy mutations that cause life-threatening muscle weakness from birth and often require mechanical assistance in breathing (Ravenscroft et al., 2015), have the same Ca\textsuperscript{2+}-sensitivity shifts as dilated cardiomyopathy mutations which are considerably less lethal (Hershberger et al., 2013). Whilst heart muscle has compensatory strategies not available in skeletal muscle to account for this difference, the small change in Ca\textsuperscript{2+}-sensitivity even in the most severe skeletal muscle disease might be indicative of a fundamental structure-based limit on changes in EC\textsubscript{50}.

Consideration of the Ca\textsuperscript{2+}-sensitivity shifts in cardiomyopathies (Tables 1 and 3) do not indicate any correlation with disease severity. Any relationship that may exist is masked by the extreme variability of Ca\textsuperscript{2+}-sensitivity shift measurements. For instance, the “severe” TNNI3 R145G HCM/RCM-linked mutation features at both extremes of the Ca\textsuperscript{2+}-sensitivity range (1.15x and 3.65x); for the 6 assays in the table the mean is 1.8x, close to the mean of all 71 HCM measurements (1.87). The same variability can be seen with other mutations where multiple values are available: ACTC E99K, n = 5, 1.24–2.45 mean 1.85; TPM1 E180G, n = 4, 1.30–2.75, mean 1.78. The second relevant observation is that the physiological modulation of cardiac muscle myofilament Ca\textsuperscript{2+}-sensitivity due to phosphorylation

### TABLE 1 | Continued

| Gene name | Mutation | wt/mutant EC\textsubscript{50} ratio | Measured in | References |
|-----------|----------|------------------------------------|-------------|------------|
| HCM       |          |                                    |             |            |
| TNNT2     | R92L     | 1.65 Fibers TG                     | Ford et al., 2012 |
| TNNT2     | R92Q     | 1.66 Fibers TG                     | Ford et al., 2012 |
| TNNT2     | R92Q     | 1.74 ATPase                        | Robinson et al., 2002 |
| TNNT2     | R92Q     | 1.94 IMA                           | Robinson et al., 2002 |
| TNNT2     | F110I    | 2.34 Fibers TG                     | Szczesna et al., 2000 |
| TNNT2     | F110I    | 1.32 Fibers TG                     | Baudenbacher et al., 2008 |
| TNNT2     | \(\Delta E160\) | 1.41 Fibers TG                   | Lu et al., 2003 |
| TNNT2     | R278C    | 2.19 Fibers TG                     | Szczesna et al., 2000 |
| TNNT2     | K280N    | 1.64 IMA                           | Messer et al., 2016b |
| TNNT2     | K280N    | 1.26 IMA (human Tn)               | Messer et al., 2016b |
| TPM1      | E62Q     | 1.21 ATPase                        | Chang et al., 2005 |
| TPM1      | A63V     | 1.91 Transfected cell              | Michele et al., 1999 |
| TPM1      | A63V     | 1.99 ATPase                        | Heller et al., 2003 |
| TPM1      | K70T     | 1.58 Transfected cell              | Michele et al., 1999 |
| TPM1      | K70T     | 2.13 ATPase                        | Heller et al., 2003 |
| TPM1      | D175N    | 1.23 IMA                           | Bing et al., 2000 |
| TPM1      | E180G    | 1.30 IMA                           | Bing et al., 2000 |
| TPM1      | E180G    | 1.63 IMA                           | Papadaki et al., 2015 |
| TPM1      | E180G    | 1.41 Transfected cell              | Michele et al., 1999 |
| TPM1      | E180G    | 2.57 ATPase                        | Chang et al., 2005 |
| TPM1      | L185R    | 2.51 ATPase                        | Chang et al., 2005 |
| TPM1      | I284V    | 1.50 Human fibers                  | Sequence et al., 2013 |

The criteria for inclusion in the table are (1) that a missense mutation has been convincingly linked to the myopathy phenotype and (2) that only direct Ca\textsuperscript{2+}-sensitivity comparisons of mutant and “normal” are included. Seventy-one independent measurements of the HCM mutation-induced change of EC\textsubscript{50} shown as EC\textsubscript{50} WT/mutant. Values range from 1.15 to 3.8-fold with a mean of 1.87 ± 0.07 (sem). Shading indicates gene studied.

### TABLE 2 | Effect of skeletal muscle gain-of-function mutations on Ca\textsuperscript{2+}-sensitivity shown as EC\textsubscript{50} WT/mutant.

| Gene name | Mutation | wt/mutant EC\textsubscript{50} ratio | Measured in | References |
|-----------|----------|------------------------------------|-------------|------------|
| ACTA1     | K326N    | 2.50 MMA                           | Jain et al., 2012 |
| TPM2      | \(\Delta E139\) | 1.51 MMA                       | Marston et al., 2013 |
| TPM2      | E181K    | 1.58 Human fibers                  | Ochala et al., 2012 |
| TPM2      | \(\Delta K7 50\%\) | 2.00 MIA                        | Mokbel et al., 2013 |
| TPM2      | \(\Delta K7\) | 2.70 Human fibers                 | Mokbel et al., 2013 |
| TPM3      | K168E    | 2.67 MIA                           | Marston et al., 2013 |
| TPM3      | K168E 50% | 1.85 MIA                           | Marston et al., 2013 |
| TPM3      | \(\Delta E224\) | 1.34 Human fibers               | Donkervoort et al., 2015 |
| TPM3      | \(\Delta E224\) | 2.2 MIA                      | Donkervoort et al., 2015 |
| TPM3      | \(\Delta 218\) | 2.5 MIA                       | Donkervoort et al., 2015 |

The mean change is 1.65 ± 0.16-fold (range 1.19–2.70). The mean change is 1.65 ± 0.16-fold (range 1.19–2.70).
of troponin I by protein kinase A has been known to be a 2–3-fold shift for many years (Salaro et al., 2008). Table 5 lists a number of recent determinations of this Ca\(^{2+}\)-sensitivity shift in several species and measured by both loaded and unloaded assays illustrating its small range. Figure 1C shows how the magnitude and distribution of measured changes is similar to the changes induced by disease-causing mutations. It would be logical to conclude that this represents the range of achievable Ca\(^{2+}\)-sensitivity shifts in cardiac muscle due to the limitations of the EC coupling system.

In principle, it should be possible to go beyond the Ca\(^{2+}\)-sensitivity limits set by EC coupling in an in vitro system where Ca\(^{2+}\) binding affinity can be much greater or much less than the native troponin. Cardiac troponin C presents extreme examples in a single molecule. Only site II binds Ca\(^{2+}\) in the physiologically relevant range (2.5 × 10\(^{-5}\) M\(^{-1}\)) and so is solely responsible for Ca\(^{2+}\)-regulation (Holroyde et al., 1980). A few amino acid changes in the EF-hand motifs results in sites that do not bind Ca\(^{2+}\) (Site I) or sites that bind Ca\(^{2+}\) 200x tighter (sites III and IV) and are permanently occupied by Ca\(^{2+}\) or Mg\(^{2+}\) (Li and Hwang, 2015). Thus, it would seem that neither a very high Ca\(^{2+}\)-sensitivity nor a very low one are able to participate in regulation. How much deviation of Ca\(^{2+}\) affinity from the norm is compatible with muscle regulation?

It is known that for mutations, the small Ca\(^{2+}\)-sensitivity changes correlate with Ca\(^{2+}\) binding affinity to thin filaments (Robinson et al., 2007). In a study of mutations induced in skeletal muscle troponin C, Davis et al. achieved a 243-fold range of Ca\(^{2+}\) binding affinities for troponin C. However, this did not translate into such a great range when Ca\(^{2+}\)-binding was measured in the presence of TnI (96–148) and caused a still smaller shift in the Ca\(^{2+}\)-sensitivity of force production (Davis et al., 2004). Thus, the most extreme Ca\(^{2+}\)-sensitizing mutation, V45Q increased TnC Ca\(^{2+}\)-binding affinity 19-fold, but the increase was only 3.1-fold when measured in the presence of the TnI peptide and Ca\(^{2+}\)-sensitivity in skinned fibers was just 2.3-fold more than wild-type. This is within the same
 FIGURE 1 | Continued

sensitivity ($\Delta pC_{a50} < 0$) = 0.255 ± 0.015. (B) Distribution of change in Ca\textsuperscript{2+} sensitivity is compared for loaded (pale blue) and unloaded (dark blue) assays of cardiac muscle regulation (data from Tables 1, 3). Unloaded assays are IVMA and ATPase, loaded assays are Fibers TG, Myofibrils TG, Fibers X, Human fibers. For decreased Ca\textsuperscript{2+} sensitivity mean unloaded $\Delta pC_{a50}$ is $-0.27 \pm 0.02$ and mean loaded is $-0.21 \pm 0.03$, $p = 0.05$. For increased Ca\textsuperscript{2+} sensitivity mean unloaded $\Delta pC_{a50}$ is $0.26 \pm 0.02$ and mean loaded is $0.021 \pm 0.02$, $p = 0.04$. (C) Distribution of change in Ca\textsuperscript{2+} sensitivity due to troponin I phosphorylation ($EC_{50}$ unphosphorylated/$EC_{50}$ phosphorylated). Data from Table 5. The mean change is $0.50 \pm 0.06$-fold ($n = 9$), $\Delta pC_{a50} = -0.30$.

TABLE 5 Ca\textsuperscript{2+} sensitivity change due to troponin I phosphorylation

| EC\textsubscript{50} | wt/mutant | Measured in | References |
|------------------|-----------|-------------|------------|
| Human failing/donor | 0.57 | IVMA | Messer, 2007; Messer et al., 2007 |
| Human failing/donor | 0.68 | Human fibers | van der Velden et al., 2003 |
| Donor uP/P | 0.34 | IVMA | Song et al., 2011 |
| Donor uP/P | 0.32 | IVMA | Bayliss et al., 2012 |
| Donor uP/P | 0.34 | IVMA | Memo et al., 2013 |
| Mouse uP/P | 0.33 | IVMA | Song et al., 2010 |
| Mouse uP/P | 0.50 | IVMA | Memo et al., 2013 |
| Mouse uP/P | 0.74 | Myofibrils | Vikhorev et al., 2014 |
| WT cTnI/cTnI-DD | 0.69 | Fibers X | Blesiadecki et al., 2007 |

Measurements were made with troponin (IVMA) or skinned muscle from human (donor) or mouse heart. The mean change is $0.50 \pm 0.06$-fold (range 0.32–0.74).

range of many HCM-causing mutations (Table 1). A similar picture emerges from Cardiac troponin C where the single regulatory Ca\textsuperscript{2+}-binding site simplifies the argument: V44Q increases Ca\textsuperscript{2+}-binding affinity to TnC 6.5-fold but increases myocyte Ca\textsuperscript{2+}-sensitivity by just 3.4-fold (Parvatiyar et al., 2010). Thus, it seems that the structure of troponin and its interactions with the rest of the thin filament does limit the consequences of a modification that increases Ca\textsuperscript{2+} binding affinity.

A slightly different situation arises when Ca\textsuperscript{2+} binding affinity is less than wild-type. Davis et al., noted that the mutations that decreased Ca\textsuperscript{2+} binding affinity the most (F26Q, 63-fold, I37Q, 24-fold and I62Q, 10-fold) could not properly regulate force in skinned fibers since they only produced about 13% of the maximal force of wild-type muscle at saturating Ca\textsuperscript{2+} concentrations. On the other hand, two less extreme mutations, M81Q and F78Q decreased Ca\textsuperscript{2+}-sensitivity whilst retaining the same maximum force production as wild type. In these cases, again, the increased Ca\textsuperscript{2+} binding affinity for TnC was substantially greater than the increased Ca\textsuperscript{2+}-sensitivity of skinned fibers (5.9x vs. 1.8x for M81Q and 8.4x vs. 4.2x for F78Q). Thus, thin filament structure seems to limit the possible effects of changes in Ca\textsuperscript{2+}-binding affinity.

It is self-evident that changing myofilament Ca\textsuperscript{2+} sensitivity will affect contractile output in muscle. It is well-established that EC\textsubscript{50} for skinned muscle fibers is about 1 µM and
that Ca\(^{2+}\)-activation of contraction is highly cooperative. Most measurements suggest a five-fold range in free Ca\(^{2+}\) concentration during a cardiac muscle contraction. Peak Ca\(^{2+}\) concentration is about 600 nM at rest and can be substantially higher during adrenergic stimulation, thus normally muscle is only partially activated (Negretti et al., 1995; Dibb et al., 2007).

**Figure 2** shows a real life example: in a mouse model of HCM (ACTC E99K) we measured both the Ca\(^{2+}\)-activation curve for myofibrils and the contractility of intact papillary muscle as well as the Ca\(^{2+}\)-transient (Song et al., 2013). Under the conditions of this experiment the Ca\(^{2+}\) transient was the same in Wild-type and ACTC E99K mouse, Ca\(^{2+}\) sensitivity was 0.8 \(\mu\)M for wild-type and 0.34 \(\mu\)M for ACTC E99K with a Hill coefficient of about 4. The increase in Ca\(^{2+}\)-sensitivity due to the ACTC E99K HCM mutation corresponds to an approximately four-fold increase in twitch force in the absence of a change in the Ca\(^{2+}\)-transient that was actually observed.

We can use this model to consider what would happen if Ca\(^{2+}\)-sensitivity changed beyond the normal range. If myofilament Ca\(^{2+}\)-sensitivity was 4 times normal, maximum force would reach close to 100%, leaving no range for it to be modulated by adrenergic agents. Moreover, it is likely that the muscle would not fully relax, since, based on the five-fold range of the Ca\(^{2+}\) transient even at the lowest Ca\(^{2+}\) level force would be 5–10%, a substantial fraction of the peak force of wild-type muscle, thus the hypercontractile phenotype would impose a major defect in relaxation, much more severe than the diastolic dysfunction associated with HCM mutations with only a 1.8-fold average Ca\(^{2+}\)-sensitivity increase.

If myofilament Ca\(^{2+}\)-sensitivity were decreased to half the normal, contractility would be very low indeed. The fact that mutations that decrease Ca\(^{2+}\)-sensitivity are not lethal and indeed in transgenic mice, may exhibit little phenotype, is probably due to a compensatory increase in the Ca\(^{2+}\)-transient (Du et al., 2007). However, this compensation may not be enough to support normal contraction in the long term, leading to DCM, the phenotype commonly associated with reduced Ca\(^{2+}\)-sensitivity.

**CONCLUSION**

The objective of this article was to confirm that Ca\(^{2+}\)-sensitivity of contractility only varies within a narrow range of three-fold above and below the normal EC\(_{50}\) at rest and to investigate why this should be. The high cooperativity of muscle activation by Ca\(^{2+}\) means there is a narrow [Ca\(^{2+}\)] range between relaxed and active muscle. It would appear that the excitation-contraction coupling machinery of the cell has limited ability to change the amplitude of the Ca\(^{2+}\)-transient or baseline [Ca\(^{2+}\)] to compensate for changes in EC\(_{50}\); thus increased Ca\(^{2+}\)-sensitivity would be limited by inability to relax and reduced Ca\(^{2+}\)-sensitivity would be limited by inability to contract. It is intriguing that the Ca\(^{2+}\)-sensitivity range of the thin filament itself is independently limited. Mutations that change Ca\(^{2+}\)-binding affinity to TnC by a large amount nevertheless only produce a small change in EC\(_{50}\) for activation of loaded or unloaded contractility in vitro. Whether this property is an evolutionary adaptation that limits the deleterious effects of mutations in thin filaments or simply fortuitous in unknown.

**AUTHOR CONTRIBUTIONS**

The author confirms being the sole contributor of this work and approved it for publication.

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**REFERENCES**

Baudenbacher, F., Schober, T., Pinto, J. R., Sidorov, V. Y., Hilliard, F., Solaro, R. J., et al. (2008). Myofilament Ca\(^{2+}\) sensitization causes susceptibility to cardiac arrhythmia in mice. *J. Clin. Invest.* 118, 3893–3903. doi: 10.1172/jci 36642

Bayliss, C. R., Jacques, A. M., Leung, M.-C., Ward, D. G., Redwood, C. S., Gallon, C. E., et al. (2012). Myofibrillar Ca\(^{2+}\)-sensitivity is uncoupled from troponin I phosphorylation in hypertrophic obstructive cardiomyopathy due to abnormal troponin T. *Cardiovasc. Res.* 97, 500–508. doi: 10.1093/cvr/cvs322

Biesiadecki, B. J., Kobayashi, T., Walker, J. S., John Solaro, R., and de Tombe, P. P. (2007). The troponin C G159D mutation blunts myofilament desensitization.
induced by troponin I Ser23/24 phosphorylation. Circ. Res. 100, 1486–1493. doi: 10.1161/01.RES.0000267744.92677.7f
Bing, W., Knott, A., Redwood, C. S., Esposito, G., Purcell, I., Watkins, S., et al. (2000). Effect of hypertrophic cardiomyopathy mutations in human cardiac α-tropomyosin (Asp175Asn and Gln180Gly) on the regulatory properties of human cardiac troponin determined by in vitro motility assay. Biochem. Biophys. Res. Commun. 272, 1489–1496. doi: 10.1006/bbrc.2000.1182
Blanchard, E., Seidman, C., Seidman, J. G., LeWinter, M., and Maughan, D. (1999). Altered crossbridge kinetics in the alphaMHC403+/+ mouse model of familial hypertrophic cardiomyopathy. Circ. Res. 84, 475–483. doi: 10.1161/01.RES.84.4.475
Brunet, N. M., Chase, P. B., Mihajlovic, G., and Schoffstall, B. (2014). Ca2+-regulatory function of the inhibitory peptide region of cardiac troponin I is aided by the C-terminus of cardiac troponin T: effects of familial hypertrophic cardiomyopathy mutations on Ca2+ sensitivity, phosphorylation kinetics and proteolytic susceptibility of troponin. J. Mol. Cell. Cardiol. 39, 754–765. doi: 10.1016/j.yjmcc.2005.05.013
Gomes, A. V., Liang, J., and Potter, J. D. (2005b). Mutations in human cardiac troponin I that are associated with restrictive cardiomyopathy affect basal ATPase activity and the calcium sensitivity of force development. J. Biol. Chem. 280, 30909–30915. doi: 10.1074/jbc.M500287200
Heller, M. J., Nilis, M., Homsher, E., and Tobacman, L. S. (2003). Cardiomyopathic troponin mutations that increase thin filament Ca2+ sensitivity and troponin I N-domain flexibility. J. Biol. Chem. 278, 41742–41748. doi: 10.1074/jbc.M303408200
Hershberger, R. E., Hedges, D. J., and Morales, A. (2013). Dilated cardiomyopathy: the complexity of a diverse genetic architecture. Nat. Rev. Cardiol. 10, 531–547. doi: 10.1038/nrcardio.2013.105
Hershberger, R. E., Pinto, J. R., Parks, S. B., Kushner, J. D., Li, D., Ludwigsen, S., et al. (2009). Clinical and functional characterization of TNNT2 mutations identified in patients with dilated cardiomyopathy. Circ. Cardiovasc. Genet. 2, 306–313. doi: 10.1161/CIRCGENETICS.108.846733
Holroyde, M. J., Robertson, S. P., Johnson, J. D., Solaro, R. J., and Potter, J. D. (1980). The calcium and magnesium binding sites on cardiac troponin and their role in the regulation of myofibrillar adenosine triphosphatase. J. Biol. Chem. 255, 11688–11693.
Jain, R. K., Jaywant, S., Squier, W., Muntoni, F., Sewry, C. A., Manzur, A., et al. (2012). Nemaline myopathy with stiffness and hypertonia associated with an ACTA1 mutation. Neurology 78, 1100–1103. doi: 10.1227/WNL.0b013e31824e8ebe
Kerrick, W. G., Kaczmierzak, K., Xu, Y., Wang, Y., and Szczesna-Cordary, D. (2009). Malignant familial hypertrophic cardiomyopathy D166V mutation in the ventricular myosin regulatory light chain causes profound effects in skinned and intact papillary muscle fibers from transgenic mice. FASEB J 23, 855–865. doi: 10.1096/fj.08-118182
Köhler, J., Chen, Y., Brenner, B., Gordon, A. M., Kraft, T., Martyn, D. A., et al. (2003). Familial hypertrophic cardiomyopathy mutations in troponin I (K183D, G203S, K206Q) enhance filament sliding. Physiol. Genomics 14, 117–128. doi: 10.1152/physiogen.00101.2002
Krüger, M., Zitrin, S., Redwood, C., Blandeck, N., James, J., Robbins, J., et al. (2005). Effects of the mutation R145L in human cardiac troponin I on the kinetics of the contraction–relaxation cycle in isolated cardiac myofibrils. J. Physiol. 564, 347–357. doi: 10.1113/jphysiol.2004.079095
Lakdawala, N. K., Delfavere, L., Redwood, C. S., Sparks, E., CIRINO, L. D., Depalma, S., et al. (2010). Familial dilated cardiomyopathy caused by an alpha-tropomyosin mutation: the distinctive natural history of sarcomeric dilated cardiomyopathy. J. Am. Coll. Cardiol. 55, 320–329. doi: 10.1016/j.jacc.2009.11.017
Lang, R., Gomes, A. V., Zhao, J., Houmans, P. R., Miller, T., and Potter, J. D. (2002). Functional analysis of a troponin I (R145G) mutation associated with familial hypertrophic cardiomyopathy. J. Biol. Chem. 277, 11670–11678. doi: 10.1074/jbc.M108912200
Li, A. Y., Stevens, C. M., Liang, B., Rayani, K., Little, S., Davis, J., et al. (2013). Familial hypertrophic cardiomyopathy related cardiac troponin C L29Q mutation alters length-dependent activation and functional effects of phosphomimetic troponin I*. PLoS ONE 8:e79363. doi: 10.1371/journal.pone.0079363
Li, M. X., and Hwang, P. M. (2015). Structure and function of cardiac troponin C (TNNC1): implications for heart failure, cardiomyopathies, and troponin modulating drugs. Gene 571, 153–166. doi: 10.1016/j.gene.2015.07.074
Lu, Q. W., Morimoto, S., Harada, K., Du, C. K., Takahashi-Yanaga, F., Miwa, Y., et al. (2003). Cardiac troponin T mutation R141F found in dilated cardiomyopathy stabilizes the troponin T-tropomyosin interaction and causes a Ca(2+) desensitization. J. Mol. Cell. Cardiol. 35, 1421–1427. doi: 10.1016/j.yjmcc.2003.09.003
Marston, S. B. (2011). How do mutations in contractile proteins cause the primary familial cardiomyopathies? J. Cardiovasc. Transl. Res. 4, 245–255. doi: 10.1007/s12265-011-9266-2
Marston, S., Momo, M., Messer, A., Papadaki, M., Nowak, K., McNamara, E., et al. (2013). Mutations in repeating structural motifs of troponin I cause gain of function in skeletal muscle myopathy patients. Hum. Mol. Genet. 22, 4978–4987. doi: 10.1093/hmg/ddt345
Martins, A. S., Parvatiyar, M. S., Feng, H.-Z., Bos, J. M., Gonzalez-Martinez, D., Vukmirovic, M., et al. (2015). In vivo analysis of troponin C knock-in (A8V) mice: evidence that TNNC1 is a hypertrophic

Frontiers in Physiology | www.frontiersin.org 7 September 2016 | Volume 7 | Article 415

[45x-84]Gafurov, B., Brenner, B., Chase, P. B., and Chalovich, J. M. (2004). The Delta 14 mutation of human cardiac troponin T enhances ATPase activity and alters the cooperative binding of S1-ADP to regulated actin. Biochemistry 43, 15276–15285. doi: 10.1021/bi048464h
Gomes, A. V., Harada, K., and Potter, J. D. (2005a). A mutation in the N-terminus of troponin I that is associated with hypertrophic cardiomyopathy affects the Ca(2+)-sensitivity, phosphorylation kinetics and proteolytic susceptibility of troponin. J. Mol. Cell. Cardiol. 39, 754–765. doi: 10.1016/j.yjmcc.2005.05.013
Gomes, A. V., Liang, J., and Potter, J. D. (2005b). Mutations in human cardiac troponin I that are associated with restrictive cardiomyopathy affect basal

[45x53]Ford, S. J., Mamidi, R., Jimenez, J., Tardiff, J. C., and Chandra, M. (2012). Increased myofilament Ca2+-sensitivity and diastolic dysfunction as

[45x32]Marston Limited Changes in Ca2+-sensitivity and diastolic dysfunction as
cardiomyopathy susceptibility gene. Circ. Cardiovasc. Genet. 8, 653–664. doi: 10.1161/CIRCGENETICS.114.000957
Martiška, M., Lehtokari, V.-L., Marston, S., Nyman, T. A., Barnerias, C., Beggs, A. H., et al. (2014). Mutation update and genotype-phenotype correlations of novel and previously described mutations in TPM2 and TPM3 causing congenital myopathy. Hum. Mutat. 35, 779–790. doi: 10.1002/humu.22554
Martiška, M., Lemola, E., Wallefeld, W., Memo, M., Donner, K., Laing, N. G., et al. (2012). Abnormal actin binding of aberrant β-tropomyosins is a molecular cause of muscle weakness in TPM2-related nemaline and cap myopathy. Biochem. J. 442, 231–239. doi: 10.1042/Bj20111030
McConnell, B. K., Singh, S., Fan, Q., Hernandez, A., Portillo, J. P., Reiser, P. J., et al. (2015). Knock-in mice harboring a Ca²⁺ desensitizing mutation in cardiac troponin C develop early onset dilated cardiomyopathy. Front. Physiol. 6:242. doi: 10.3389/fphys.2015.00242
Memo, M., Leung, M.-C., Ward, D. G., dos Remedios, C., Morimoto, S., Zhang, L., et al. (2013). Mutations in thin filament proteins that cause familial dilated cardiomyopathy uncouple troponin I phosphorylation from changes in myofibrillar Ca²⁺-sensitivity. Cardiovasc. Res. 99, 65–73. doi: 10.1093/cvr/cvt071
Messer, A. (2007). Structural and Functional Polymorphisms of Troponin in Failing Heart. Ph.D., Thesis NHLI London, London. 343.
Messer, A., Bayliss, C., El-Mezgueldi, M., Redwood, C., Ward, D. G., Leung, M.-C. et al. (2016b). Mutations in troponin T associated with Hypertrophic Cardiomyopathy increase Ca²⁺-sensitivity and suppress the modulation of Ca²⁺-sensitivity by troponin I phosphorylation. Arch. Biochem. Biophys. 601, 113–120. doi: 10.1016/j.abb.2016.03.027
Messer, A. E., Jacques, A. M., and Marston, S. B. (2007). Troponin phosphorylation and regulatory function in human heart muscle: dephosphorylation of Ser23/24 on troponin I could account for the contractile defect in end-stage heart failure. J. Mol. Cell. Cardiol. 42, 247–259. doi: 10.1016/j.yjmcc.2006.08.017
Messer, A. E., Papadaki, M., Vikhorev, P. G., Sebali, Y., El-Mezgueldi, M., Daley, A., et al. (2016a). *Primary effects of HCM mutations in humans and cats. Biophys. J. 110, 123a–124a. doi: 10.1016/j.bpj.2015.11.713
Michele, D. E., Albayya, F. P., and Metzger, J. M. (1999). Direct, convergent hypersensitivity of calcium-activated force generation produced by hypertrophic cardiomyopathy mutant alpha-tropomyosins in adult cardiac myocytes. Nat. Med. 5, 1413–1417. doi: 10.1038/70990
Mirza, M., Marston, S., Willott, R., Ashley, C., Mogensen, J., McKenna, W., et al. (2005). Dilated cardiomyopathy mutations in three thin filament regulatory proteins result in a common functional phenotype. J. Biol. Chem. 280, 28498–28506. doi: 10.1074/jbc.M412281200
Mokbel, N., Ikovski, B., Kreissl, M., Memo, M., Jeffries, C. M., Marttila, M., et al. (2013). K dél is a common TPM2 gene mutation associated with nemaline myopathy and raised myofibre calcium sensitivity. Brain 136, 494–507. doi: 10.1093/brain/aws348
Negretti, N., Varro, A., and Eissner, D. A. (1995). Estimate of net calcium changes in myofibrillar Ca²⁺-sensitivity by troponin I phosphorylation from cardiac troponin I to C. FIBBS 277, 40710–40716. doi: 10.1042/0161-202x.M203446200
Schmidtmann, A., Lindow, C., Villard, S., Heuser, A., Mügge, A., Gessler, R., et al. (2005). Cardiac troponin T-L29Q, related to hypertrophic cardiomyopathy, hinders the transduction of the protein kinase A dependent phosphorylation signal from cardiac troponin I to C. FIBBS J. 272, 6087–6097. doi: 10.1111/j.1472-4458.2005.00501.x
Sequeira, V., Wijnker, P. J., Nijenkamp, L. L., Kuster, D. W., Najafi, A., Wijtsa-Paalberends, E. R., et al. (2013). Perturbed length-dependent activation in human hypertrophic cardiomyopathy with missense sarcomeric mutations. Circ. Res. 112, 1491–1505. doi: 10.1161/CIRCRESAHA.111.300436
Soloro, R. J., Rosevear, P., and Kobayashi, T. (2008). The unique functions of cardiac troponin I in the control of cardiac muscle contraction and relaxation. Biochem. Biophys. Res. Commun. 369, 82–87. doi: 10.1016/j.bbrc.2007.12.114
Song, W., Dyer, E., Stuckey, D., Copeland, O., Leung, M., Bayliss, C., et al. (2011). Molecular mechanism of the Glu99lys mutation in cardiac actin (ACTC gene) that causes apical hypertrophy in man and mouse. J. Biol. Chem. 286, 27582–27593. doi: 10.1074/jbc.M111.252320
Song, W., Dyer, E., Stuckey, D., Leung, M.-C., Memo, M., Mansfield, C., et al. (2010). Investigation of a transgenic mouse model of familial dilated cardiomyopathy. J. Mol. Cell. Cardiol. 49, 380–389. doi: 10.1016/j.yjmcc.2010.05.009
Song, W., Vikhorev, P. G., Kashyap, M. N., Rowlands, C., Ferenczi, M. A., Lunardi, J., et al. (2004). Differential cross-bridge kinetics of FHC myosin mutations induce thin filament dysfunction via distinct physiological mechanisms. J. Mol. Genet. 33, 2095–2105. doi: 10.1152/ajpheart.00951.2012
Szczesna-Cordary, D., Guzman, G., Ng, S. S., and Zhao, J. (2004). Familial hypertrophic cardiomyopathy-linked alterations in Ca²⁺ binding of human cardiac myosin regulatory light chain affect cardiac muscle contraction. J. Biol. Chem. 279, 624–630. doi: 10.1074/jbc.275.1.624
Szczesna, D., Zhang, R., Zhao, J., Jones, M., Guzman, G., and Potter, J. D. (2000). Altered regulation of cardiac muscle contraction by troponin T mutations that cause familial hypertrophic cardiomyopathy. J. Biol. Chem. 275, 624–630. doi: 10.1074/jbc.275.1.624
Takahashi-Tanaga, F., Morimoto, S., Harada, K., Minakami, R., Shiroya, F., Ohta, M., et al. (2001). Functional consequences of the mutations in human cardiac troponin I gene found in familial hypertrophic cardiomyopathy. J. Mol. Cell. Cardiol. 33, 2095–2107. doi: 10.1006/jmcc.2001.1473
of contractile proteins. *Cardiovasc. Res.* 57, 37–47. doi: 10.1016/S0008-6363(02)00606-5

Venkatraman, G., Gomes, A. V., Kerrick, W. G., and Potter, J. D. (2005). Characterization of troponin T dilated cardiomyopathy mutations in the fetal troponin isoform. *J. Biol. Chem.* 280, 17584–17592. doi: 10.1074/jbc.M409337200

Vikhorev, P. G., Song, W., Wilkinson, R., Copeland, O., Messer, A. E., Ferenczi, M. A., et al. (2014). The dilated cardiomyopathy-causing mutation ACTC E361G in cardiac muscle myofibrils specifically abolishes modulation of Ca(2+) regulation by phosphorylation of troponin I. *Biophys. J.* 107, 2369–2380. doi: 10.1016/j.bpj.2014.10.024

Warren, C. M., Karam, C. N., Wolska, B. M., Kobayashi, T., de Tombe, P. P., Arteaga, G. M., et al. (2015). A green tea catechin normalizes the enhanced Ca^{2+} sensitivity of myofilaments regulated by a hypertrophic cardiomyopathy associated mutation in human cardiac troponin I (K206I). *Circ. Cardiovasc. Genet.* 8, 765–773. doi: 10.1161/CIRCUGENETICS.115.001234

Yuan, C.-C., Muthu, P., Kazmierczak, K., Liang, J., Huang, W., Irving, T. C., et al. (2015). Constitutive phosphorylation of cardiac myosin regulatory light chain prevents development of hypertrophic cardiomyopathy in mice. *Proc. Natl. Acad. Sci. U.S.A.* 112, E4138–E4146. doi: 10.1073/pnas.1505819112

Yuen, M., Cooper, S. T., Marston, S. B., Nowak, K. J., McNamara, E., Mokbel, N., et al. (2015). Muscle weakness in TPM3-myopathy is due to reduced Ca^{2+}-sensitivity and impaired acto-myosin cross-bridge cycling in slow fibres. *Hum. Mol. Genet.* 24, 6278–6292. doi: 10.1093/hmg/ddv334

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