

The Degree of Activation of Cardiac Muscle Depends on Muscle Length

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The Frank-Starling mechanism of the heart¹⁻³ can be described as the relationship between force and length of cardiac muscle. With the advent of the cross bridge theory of muscle contraction^{4,5} and Gordon and collaborator's classical study⁶ describing the fitting of this theory to the force-length relationship in skeletal muscle, several investigators started to study this relationship in cardiac muscle.

The force-length relation in cardiac muscle is observed in a small variety of sarcomere lengths, between approximately 1.8 μm and 2.3 μm . This region corresponds to the ascending limb of the force-length relation of skeletal muscle⁶. The force levels of the myocardium vary from zero at 1.8 μm to maximal force values at 2.3 μm . This large variation in force results in a very steep force-length relation, especially when compared with this relation in skeletal muscle (fig. 1). To get an idea, when the tension developed by the myocardium at different sarcomere lengths is normalized in relation to its maximal force (F_{max}) at the point where maximal length (L_{max}) occurs, the developed tension is approximately 10-15% when the myocardium is measured at 80% L_{max} . Yet in skeletal muscle, normalized force is approximately 80-85% of F_{max} ⁷ under the same conditions (fig.1).

This difference between skeletal and cardiac muscle shows that the force-length relationship in the myocardium are not a simple function of the degree of superposition of actin and myosin filaments. Because their lengths are similar in both muscles, other factors must be involved in the muscle force-length relationship. During recent years, this difference has been attributed to the dependence of muscle activation on sarcomere length⁸.

Muscle activation has been used collectively in the literature to refer to various processes able to initiate muscle contraction, modifying the muscle state from "inactive" to "active". Thus, muscle activation has been associated with the frequency of muscle stimulation or membrane action potentials with intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$, or the occupation of the protein troponin C (TnC) by Ca^{2+} . In this article, the term muscle contraction will be utilized to

describe the proportion of TnC associated with Ca^{2+} . The association $\text{TnC}/\text{Ca}^{2+}$ represents a fundamental event in muscle contraction; therefore, the sensitivity of TnC to Ca^{2+} will be discussed in greater detail. This choice, furthermore, comes from studies shewing that myocardial force changes due to muscle length are unrelated to $[\text{Ca}^{2+}]_i$ ^{9,10}.

The present article contains a review of studies related to the dependence of force and, more importantly, of the process of myocardial muscle activation on muscle length. In particular, the article intends to investigate mechanisms responsible for the dependence of the sensitivity of filaments to Ca^{2+} on muscle length, as proposed in the literature.

Dependence of the sensitivity of filaments to Ca^{2+} on muscle length - Evidence that the activation of the myocardium is dependent on muscle length is found in studies demonstrating that inotropic interventions (for example, increased frequency of muscle stimulation) induce a displacement of the force-length relationship of the myocardium to the left. As a result, the slope of the force-muscle length relation resembles the relationship observed in skeletal muscle (fig.1)^{7,11,12}.

Three main lines of research have been used for the evaluation of the dependence of the activation and sensitivity of the Ca^{2+} regulatory system on muscle length: 1) studies using intracellular Ca^{2+} markers and rapid shortening of intact myocardial fibers; 2) studies using membrane-free fibers; 3) studies with isotope protein markers.

Studies measuring Ca^{2+} following abrupt fiber length changes are used to evaluate the affinity of TnC for Ca^{2+} . In these studies, fluorescent Ca^{2+} marker compounds are introduced into cardiac cells, and, following adequate stimulation, they supply information about the intracellular quantity of Ca^{2+} . These studies confirm that a reduction in the $\text{TnC}/\text{Ca}^{2+}$ affinity results in higher free Ca^{2+} , because less Ca^{2+} is associated with TnC. This in turn would induce less muscle activation (according to the concept used in this article).

Different authors have demonstrated that an abrupt shortening of the intact cardiac muscle during the final stages of contraction results in decreased muscle tension, accompanied by increased levels of Ca^{2+} . This observation suggests a decreased $\text{TnC}/\text{Ca}^{2+}$ association, i.e., the affinity of TnC for Ca^{2+} decreases when the muscle is shortened^{10,13-15}.

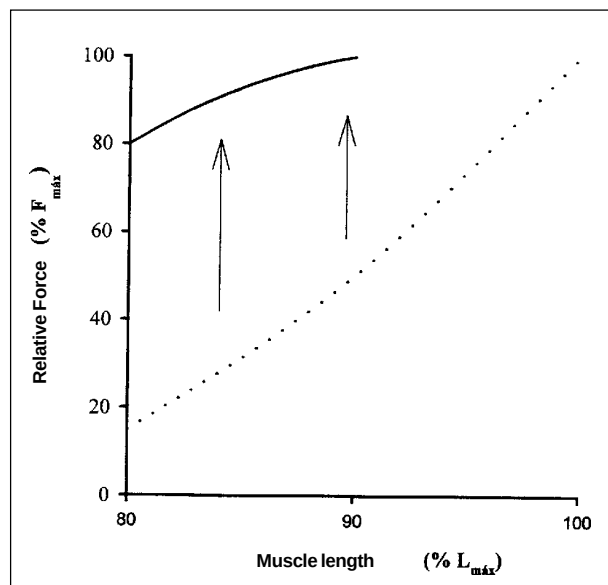


Fig. 1 - Schematic illustration of the force-length relationship of skeletal (solid line) and cardiac (broken line) muscle from 80% L_{max} on. At this point, skeletal muscle produces 80-85% of its F_{max} , while cardiac muscle produces 10-15%. At 90% L_{max} , skeletal muscle produces force close to F_{max} , while cardiac muscle produces 50% F_{max} . This is represented by an increased slope of the ascending limb of the force/length relationship in myocardial muscle (the region of normal operation of the myocardium) when compared with skeletal muscle.

Studies using membrane-free fibers analyze myocardial cells without the sarcolemma enveloping the proteins responsible for muscle contraction. In this type of preparation, muscle contraction is initiated by the addition of Ca^{2+} to fibers kept in a liquid medium that also contains substances required to maintain the viability of the experiments, e.g., glucose. In this way, the investigator, by changing extracellular Ca^{2+} concentration, is able to establish force/ Ca^{2+} relationships under different conditions (fig. 2) and can control the activation induced by Ca^{2+} .

The relationship force/ Ca^{2+} is extremely efficient for the study of cardiac muscle because it permits the investigation of the sensitivity of the regulatory system to Ca^{2+} . According to this relationship, an increase in Ca^{2+} is associated with an increase in the force developed by the myocardium up to a given plateau. If the amount of Ca^{2+} necessary to produce a given force is decreased, the force/ Ca^{2+} relation is displaced towards the left (fig. 2), and the sensitivity of the system to Ca^{2+} increases.

In this context, it has been demonstrated that when the response is evaluated in long of sarcomeres, the amount of Ca^{2+} required to obtain a given force is decreased^{16,17}. For example, in one of these studies, Hibberd and Jewell¹⁶ demonstrated that the amount of Ca^{2+} necessary for the force to reach 50% of F_{max} in a sarcomere set to a length of $\sim 2.5 \mu m$ was significantly smaller than when the sarcomere was set to a length of $\sim 1.9 \mu m$. In agreement with these results, Kentish et al¹⁷ demonstrated that each increase in sarcomere length was followed by an increase in muscle force running parallel to a slight shift to the left of the force/ Ca^{2+} relationship, signifying that a lesser amount of Ca^{2+} was necessary for the production of the same force.

Finally, some studies use specific isotope markers to analyze the degree of the association TnC/Ca^{2+} and to relate it to muscle length^{18,19}. These isotopes bind to specific TnC protein molecules, supplying information about the degree of the TnC/Ca^{2+} association. In one of these studies¹⁹, the authors, using membrane-free bovine ventricle fibers tagged with these isotopes, demonstrated a strong association of Ca^{2+} to TnC during force generation. However, this association was directly related to muscle length; a reduction was observed in shorter sarcomeres. These results agree with previously cited studies in which the dependence of muscle length on Ca^{2+} sensitivity is related to TnC.

The major question to be answered is: how does TnC, a protein subunit associated with the binding of Ca^{2+} and Mg^{2+} , receive information regarding structural changes in muscle length? To answer this question, different lines of investigation and hypotheses are presently under investigation. Some authors suggest that TnC itself might be the sensor of changes in muscle length²⁰⁻²². This hypothesis is based on molecular differences between TnC of cardiac and skeletal muscle, which might be associated with the difference in the force-length relationship in both muscles. However, several studies have refuted this hypothesis^{23,24}. In one of them, Moss et al²³ characterized the relation between force produced and activation induced by Ca^{2+} , at sarcomere lengths of $2.32 \mu m$ and $1.87 \mu m$ in membrane-free rabbit skeletal muscle fibers. Measurements were performed prior to and following substitution of TnC by cardiac muscle TnC to determine whether this was the main reason for the differences in force-length relationships of skeletal and cardiac muscle. Following a >95% substitution of skeletal muscle TnC by cardiac muscle TnC, no significant changes in

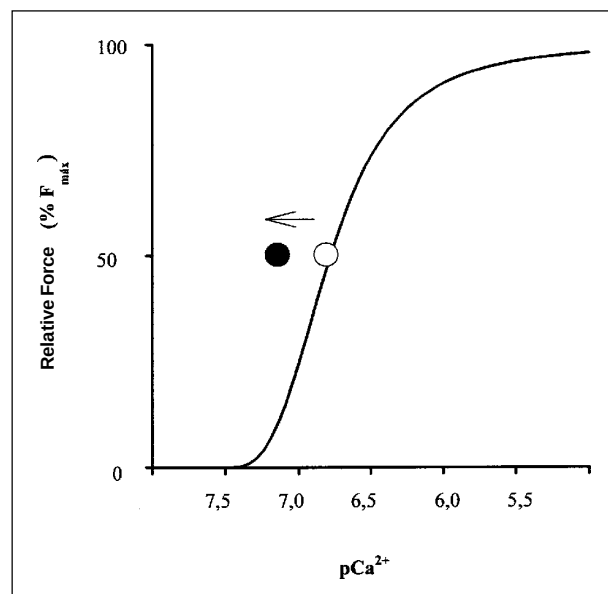


Fig. 2 - The force/ Ca^{2+} relationship. $[Ca^{2+}]$ is represented by $pCa^{2+} = (-\log_{10} [Ca^{2+}])$. The figure demonstrates that the amount of Ca^{2+} required for the production of force is decreased when the force/ pCa^{2+} relationship is displaced to the left (arrow directed from the empty circle to the full circle). This displacement is an indication of an increased sensitivity of the muscle system to Ca^{2+} .

Ca^{2+} sensitivity at the investigated lengths were observed. Therefore, TnC is not the major mechanism responsible for the dependence of the sensitivity of the muscle system on muscle length.

The hypothesis that is being successfully tested is that the association of cross bridges of myosin with actin and consequent force generation is responsible for the dependence of the degree of activation on muscle length^{18,19,25-29}. The work and justification related to this hypothesis are presented below.

Force-length relationship of cardiac muscle and the association of cross bridges with actin - When cardiac muscle is lengthened at the ascending limb of the force-length relationship, an increased superposition of actin and myosin filaments takes place. This leads to an increased probability of interaction between cross bridges and actin, and consequently increased force generation. Strong lines of evidence exist in favor of a system in which this association increases sensitivity to Ca^{2+} of the system regulating force and, consequently, muscle activation.

This hypothesis has been tested in detail in the elegant studies of Fuchs et al^{18,19,26,27}. Hofmann and Fuchs¹⁸ measured TnC/ Ca^{2+} association in membrane-free cardiac fibers using isotopes, as previously explained. As expected, results confirmed that TnC/ Ca^{2+} association depends on muscle length over the 1.7 μm to 2.4 μm range. In some experiments, the authors used a substance similar to phosphate (P_i), sodium vanadate (V_i), which acts as an ATPase enzyme, depressing the interaction of cross bridges with actin, forming a stable myosin • ADP • V_i complex. When V_i was used, the relationship between TnC/ Ca^{2+} and sarcomere length was depressed, and the degree of muscle activation was not dependent on sarcomere length. Therefore, the dependence of the affinity of Ca^{2+} to TnC on muscle length would in reality be dependent of the number of cross bridges associated with actin.

Other evidence of the association of cross bridges with the affinity of TnC to Ca^{2+} is provided by the study of Saeky et al³⁰, who used intact cardiac muscle fibers. In this work, fibers were injected with Ca^{2+} markers to perform rapid muscular shortening maneuvers, as already explained. In one set of experiments, extra Ca^{2+} was not detected when the fiber was shortened starting from a relaxed muscle state (without force production). In another group of experiments, the cycle of cross bridges was blocked by a specific chemical substance (2,3 butanedione monoxime). It was noted that, changes in muscle length did not result in the appearance of extra Ca^{2+} in the intracellular space, even though force had decreased to a considerable extent. In other words, when the association between cross bridges and actin was blocked by a state of muscle relaxation or by a specific chemical substance, the association between TnC/ Ca^{2+} did not depend on muscle length.

This leads to the conclusion that the increased sensitivity of the Ca^{2+} regulatory system induced by an increase in sarcomere length is related to an increased number of

actin-associated cross bridges, both in membrane-free cardiac fibers and in cardiac fibers with intact membranes.

Cross bridges and regulation of troponin C activity -

Although association of cross bridges, force, and sensitivity of TnC to Ca^{2+} are related events, need exists to study the nature of these relationships. In other words, how does the association of crossed bridges of myosin with actin increase the affinity of TnC to Ca^{2+} and, consequently, the sensitivity of the muscle system to Ca^{2+} ? Studies in which TnC was tagged with fluorescent probes by substitution of specific molecules at some of its regulatory domains furnish some evidence on this issue³¹⁻³³.

Hannon et al³³ used cardiac TnC tagged with fluorescent probes, to furnish specific information concerning the structure of TnC resulting from its association with Ca^{2+} . The authors measured sensitivity of responses to Ca^{2+} of the muscle system related to the association of cross bridges to link them to alterations of the structure of TnC. They observed that the association of cross bridges with actin caused conformation changes in TnC, and that this alteration was accompanied by an increased sensitivity of the muscle system to Ca^{2+} .

Along the same line of investigation, Liou and Fuchs³¹ tagged two TnC residues in bovine cardiac fibers with reactive compounds. By measuring the fluorescent signals of the compounds, they observed that cross bridges "in rigor" and cyclic cross bridges had different effects on the conformation of TnC, suggesting that the mechanisms by which cross bridges affect TnC involve molecular configuration.

These two studies offer an explanation for the mechanism responsible for the dependence of Ca^{2+} sensitivity on muscle length, by stating that an increase in muscle length induces alterations in the molecular conformation of TnC, resulting in an increase in its affinity for Ca^{2+} .

Evidence for other mechanisms responsible for the dependence on muscle length of myocardial activation -

Although evidence pointing to mechanisms involving cross bridges as the phenomenon responsible for the dependence of force and filament sensitivity to Ca^{2+} on muscle length, this hypothesis has one obvious difficulty: sensitivity to Ca^{2+} is increased in membrane-free cardiac fibers³⁴ when these are lengthened in the descending limb of the force-length relation, a region where the potential for myosin-actin interactions is decreased.

The hypothesis provided in the literature is that besides the association of cross bridges, force and TnC/ Ca^{2+} affinity, changes in sarcomere length *per se* are responsible for alterations in the muscle system sensitivity to Ca^{2+} . Studies using X-ray diffraction have demonstrated that actin and myosin filaments are positioned closer when the muscle is lengthened and muscle volume remains unmodified^{35,36}. It is reasonable, therefore, to suppose that the probability of interactions between cross bridges and actin would be increased in this situation, where the distance for

association decreases. This possibility of an increased interaction between the filaments would increase force at a given concentration of Ca^{2+} thus increasing the sensitivity of the muscle system to this cation.

Along this line of investigation, different authors have studied the effects of osmotic compression of myosin and actin filaments, using a high molecular weight polymer dextran T-5000, polyvinylpyrrolidone-40. This compound does not penetrate the space between the actin and myosin filaments and causes lateral approximation between them. Some authors^{26,37} observed that following the use of this substance, sensitivity to Ca^{2+} in cardiac muscle was significantly increased.

Wang and Fuchs²⁶ specifically investigated the hypothesis that alterations in the space between myosin and actin filaments contribute to the dependence on muscle length of myocardial sensitivity to Ca^{2+} . These authors studied simultaneously the effect of osmotic compression (Dextran

T-5000, 5 and 10%), sarcomere length (1.7 μm to 2.3 μm), and the degree of $\text{TnC}/\text{Ca}^{2+}$ association. The results are convincing: the use of 5% dextran on a membrane stabilized at a sarcomere length of 1.7 μm resulted in a 13% reduction in the diameter of the muscle fiber, equivalent to the situation in which the sarcomere is lengthened to 2.3 μm . Most importantly, this intervention was accompanied by a significant increase in muscle sensitivity to Ca^{2+} [an alteration of -0.25 pCa^{2+}] (fig. 2) and in the association $\text{TnC}/\text{Ca}^{2+}$ at activation levels between 6.0 and 5.0 pCa^{2+} .

Conclusion - The studies reviewed in this article suggest that the dependence of sensitivity to Ca^{2+} on muscle length is an associative result of the interaction of myosin cross bridges and actin, which induces alterations of the $\text{TnC}/\text{Ca}^{2+}$ affinity, and of changes in the space between the filaments, which increases the probability of myosin/actin interaction.

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