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Exercise-induced changes in calcium handling in left ventricular cardiomyocytes

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1. ABSTRACT

Regular exercise training results in beneficial adaptation of the heart by improving its contractile capacity. This has important consequences for both healthy individuals and those with depressed myocardial function, e.g. heart failure. Studies combining experimental animal models of exercise training and heart failure with biophysical and biochemical characterization of heart function have extended our understanding of how exercise training improves cardiac contractile function at the cellular level. Exercise training improves the strength of contraction and increases the rates of shortening and relengthening of cardiomyocytes. Myocardial force production and power output in heart cells studied under loaded conditions is also increased. These changes are associated with faster rise and decay of the intracellular calcium transient and improved myofilament sensitivity to calcium. Translated to global cardiac function, these cellular changes explain exercise training-induced improvements in left ventricular systolic and diastolic function. In particular, exercise training is able to restore depressed contractility and calcium cycling associated with heart failure, to a value comparable to healthy individuals.

2. INTRODUCTION

One of the benefits of regular aerobic endurance exercise training is improved pump function of the heart. Since cardiac function is a major determinant of aerobic exercise capacity and fitness (1), a demonstration of a close link between maximal oxygen uptake ($\dot{V}O_{2max}$), as a measure of aerobic fitness, and stroke volume, as a measure of cardiac pump function, comes to no surprise (2, 3). Also, analytical modeling approaches (4-7) and the fact that the main drop in the partial pressure of oxygen occurs between the pulmonary and skeletal muscle capillaries (8) suggest that cardiac function is rate-limiting to aerobic exercise. At the whole-heart level, better pump function in exercise-trained versus untrained subjects is manifested as increased cardiac output and stroke volume in humans (2, 3, 9), but improved atrio-ventricular plane displacement has also been suggested (10), though not uniformly (9). Exercise training-induced improvement in systolic and diastolic function of the heart (2, 3, 11, 12) and morphological changes, such as increased cardiac dimensions and mass (13, 14), constitute the concept of the athlete's heart. In summary, improving the heart's ability to pump blood into the vascular system for transfer to the peripheral skeletal

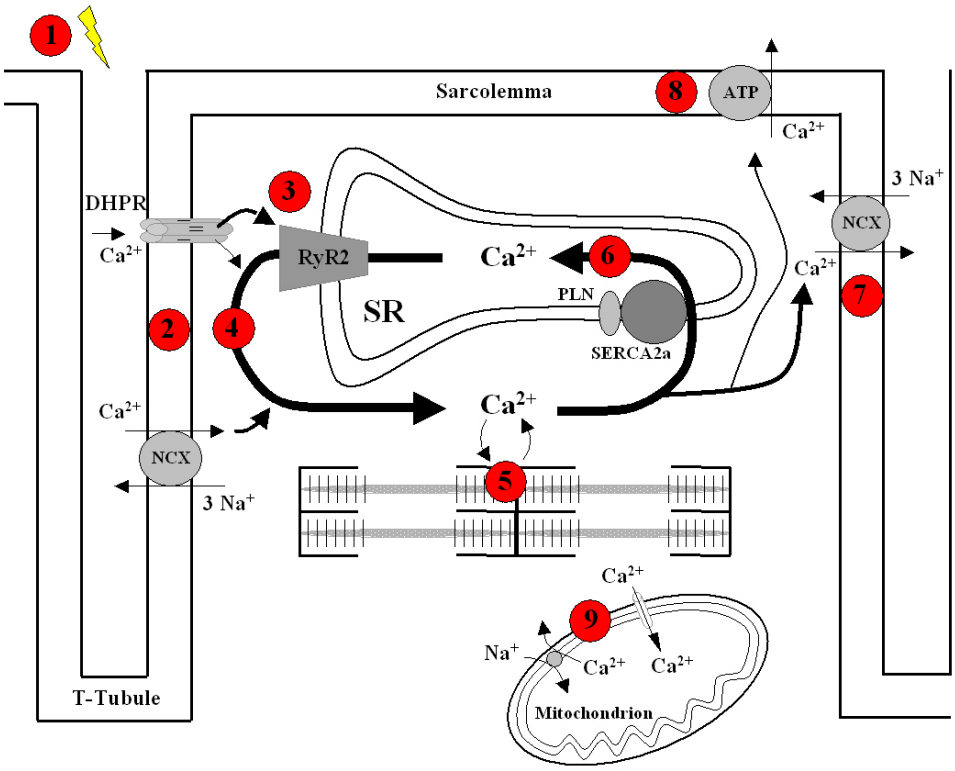

Figure 1. Excitation-contraction coupling in left ventricular cardiomyocytes. DHPR: L-type Ca^{2+} channel; NCX; Na^+/Ca^{2+} exchanger; T-Tubule: Transverse tubule; RyR2: Ryanodine receptor-2; SR: Sarcoplasmic reticulum; PLN: Phospholamban; SERCA2a: SR Ca^{2+} ATPase-2a. The numbers refer to the chain of events during the excitation-contraction coupling; 1: depolarization of the plasma membrane and T-Tubule; 2: opening of the DHPR and NCX in reverse mode; 3: opening of the RyR2; 4: release of Ca^{2+} from the SR; 5: binding of Ca^{2+} to Troponin C in the myofilaments; 6: re-uptake of Ca^{2+} into the SR through the SERCA2a; 7: Ca^{2+} transport out of the cell through the forward mode NCX; 8: Ca^{2+} transport out of the cell through the plasma membrane Ca^{2+} ATPase; and 9: mitochondrial Ca^{2+} uptake. Modified with permission from (21).

muscles hence enables exercise at higher work loads than before engaging in regular exercise programs.

Studies of cardiac function in humans have largely relied upon techniques such as echocardiography and Doppler ultrasound recordings to image cardiac wall motion and flow velocities (9, 10, 15), indirect measures of cardiac output by e.g. acetylene rebreathing (16), thermodilution techniques (17), oxygen uptake ($\dot{V}O_2$) coupled to arterial-venous oxygen difference (Fick method; 17), or by modeling the different properties along the transfer pathway of oxygen from ambient air to skeletal muscle fiber mitochondria (6, 7). Studying cardiac function in humans during maximal or near-maximal exercise effort would by nature be the gold standard, but is not without problems. There are technical difficulties with echocardiography in subjects exercising with maximum full-body effort and high heart rates, and ethical concerns with maximal exercise in cardiovascular patients with compromised cardiac function. Also, clinical studies can today only bring us up to a certain point on the underlying myocardial biology, especially regarding cellular and molecular regulation. In the following, we will review the cellular basis for improved myocardial contractile function and Ca^{2+} cycling after regular exercise training.

3. CARDIOMYOCYTE EXCITATION-CONTRACTION COUPLING AND CALCIUM CYCLING

The rapid, transient increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) evokes global cell shortening and provides thus the cellular mechanism for cardiac contractions on a beat-to-beat basis. Conversely, the decay of the cytosolic $[Ca^{2+}]_i$ after the systolic rise causes diastolic relengthening (relaxation) of the cardiomyocyte. Details of the subcellular events regulating Ca^{2+} induced Ca^{2+} release are illustrated in Figure 1. Depolarization of the sarcolemma and the transverse tubules during the action potential allows a small entry of Ca^{2+} through the dihydropyridine receptor (DHPR; L-type Ca^{2+} channel) and through reverse mode Na^+/Ca^{2+} exchanger (NCX). The resulting localized $[Ca^{2+}]_i$ increase in the cleft between the transverse tubule and the sarcoplasmic reticulum (SR) stimulates the ryanodine receptor-2 (RyR2) to release µM-order of Ca^{2+} from the SR. The close proximity of the inward Ca^{2+} channels in the transverse tubules and the RyR2 complex in the SR secures a fast and efficient coupling, whereas RyR2-embedded proteins as FKBP12.6 regulate the open probability of the RyR2 to prevent diastolic leakage. Localized Ca^{2+} release (Ca^{2+} sparks)

coordinates and generates the $[Ca^{2+}]_i$ transient that induces contraction (18-20). Post-contraction, the main bulk of Ca^{2+} is re-sequestered (Ca^{2+} decay) by the SR Ca^{2+} ATPase-2a (SERCA2a), which activity is closely regulated by phospholamban (PLN). Thus, SERCA2a recharges the SR Ca^{2+} load, whereas normal mode NCX translocates Ca^{2+} out of the cell to the extracellular space. A minor degree of removal of cytosolic Ca^{2+} occurs also through the plasma membrane Ca^{2+} ATPase and the mitochondrial Ca^{2+} uniporter. In rat ventricular cardiomyocytes, ~92% of the global Ca^{2+} transient is due to SR Ca^{2+} cycling (RyR2 release and SERCA2a uptake), whereas the L-type Ca^{2+} channel and reverse-mode NCX during systole and forward-mode NCX in diastole constitutes ~7% of the Ca^{2+} transient, with only ~1% left for the sarcolemmal Ca^{2+}-ATPase and the mitochondrial Ca^{2+} uniporter. Although there is a certain degree of species-dependence to the quantitative distribution; rabbits and large mammals inclusive humans having a longer action potential that prolongs the activation of the L-type Ca^{2+} channel and the NCX (~30%), the qualitative mechanisms are similar (21, 22). During steady-state cycles, the amount of Ca^{2+} crossing the particular membranes during systole equals that of diastolic crossing, in order to maintain an extracellular to intracellular ion balance.

3.1. Intracellular calcium and cardiomyocyte contractility

Evidence of $[Ca^{2+}]_i$ associating closely with contractility and the extent of shortening in the cardiomyocyte has come from several experimental approaches. First, experimentally reducing $[Ca^{2+}]_i$ leads to an immediate reduction in fractional shortening (23). Secondly, contractile dysfunction is tightly coupled to abnormal excitation-contraction coupling and depressed Ca^{2+} handling (18, 24-28), and finally, adenoviral gene therapy restoring low SERCA2a levels in cardiomyocytes also corrects abnormal Ca^{2+} handling and contractile dysfunction (29, 30), whereas targeted overexpression of SERCA2a enhances contractility (31, 32). However, a close intrinsic control is needed to maintain balanced Ca^{2+} cycling (33).

3.2. Calcium induces the myofilament cross-bridge cycle

The contractile apparatus of the cardiomyocyte consists of repeating ~1.6-2.1 μm long sarcomeres that are interconnected at the z-lines (34). The sarcomere units are composed of actin and myosin filaments, tropomyosin and the troponin complex that constitute the contractile machinery, and proteins that make up the support and regulatory network, such as titin, myosin binding protein C, M-protein, myomesin, nebulin and nebulette, and α- and β-actinin (21, 22). When Ca^{2+} binds to troponin C, the complex changes conformation and troponin I moves to allow actin-myosin coupling, while troponin T attaches the complex to tropomyosin. After the Ca^{2+}-induced conformational change, myosin heads bind to actin and flex to slide the filaments in opposite directions to shorten the sarcomere; hence, the process is termed the sliding filament model. Upon release of Ca^{2+} from troponin C, the sarcomere returns to its innate resting status and awaits the next contractile cycle.

Cardiomyocyte contractility is measured as the magnitude of shortening normalized to diastolic cell length; fractional shortening, whereas the velocities of contraction and relaxation are measured by the time from stimulus to half- or peak shortening, or as in the case of relaxation; the time from peak shortening to half-relaxation. Such measurements have proven to effectively characterize cellular contractile adaptations of the heart to exercise training (35-37), to heart failure (25, 27, 38-40), and to other potentially detrimental conditions and diseases (41).

3.3. Myofilament calcium sensitivity

A change in the extent of cell shortening may not always correspond to altered bio-availability of Ca^{2+}. An additional mechanism for altered contractility in the cardiomyocyte is that the sensitivity of the myofilaments to activation by Ca^{2+} may also change, e.g. an increase in myofilament Ca^{2+} sensitivity would lead to a greater isometric tension generation at the same $[Ca^{2+}]_i$. The biological basis for altered Ca^{2+} sensitivity is not well defined yet, but modification in the contractile machinery, e.g. troponin subunits and myosin light chains have been proposed (42-45). Another plausible explanation may be changed cooperation of actin and myosin or other contractile proteins, independent of Ca^{2+}. Also, an inter-dependence between intracellular pH (pH_i) and contractile function has also been established in healthy hearts several decades ago, at least partly because low pH_i reduces Ca^{2+} binding to troponin C (46). In both intact and skinned cardiomyocytes, a drop in pH_i leads to a rightward shift in the Ca^{2+}-force curve, indicating reduced Ca^{2+} sensitivity (47). The proposed mechanism for pH-dependence of contractility is that intracellular protons compete with Ca^{2+} ions for binding to troponin C, and thus shift the dependence of actomyosin ATPase and tension generation to higher $[Ca^{2+}]_i$. Since both compensatory and causative roles to contractile dysfunction in response to myocardial infarction and heart failure have been reported (25, 27, 39, 48-50), despite improved clinical outcome of Ca^{2+} sensitizers (51), the exact physiological roles of myofilament Ca^{2+} sensitivity and changes thereof remain unsettled.

4. CARDIOMYOCYTE CONTRACTILITY AND EXERCISE TRAINING

From clinical trials, it is known that one of the central features with regular exercise training is improved systolic and diastolic function of the heart (2, 3, 12). In order to study the underlying mechanisms of improved cardiac contractile performance after regular exercise training, several rodent exercise models have been developed, ranging from voluntary wheel running, to involuntary swim and treadmill running with fixed or relative intensities protocols (36, 37, 52-59). These exercise models have provided evidence that aerobic endurance exercise training increases end-diastolic volume because of an increased diastolic filling time due to lowered heart rate during submaximal exercise, increased submaximal and maximal stroke work, increased ejection fraction, and greater mid-wall fractional shortening in perfused working rat heart experiments (11, 60). Greater isometric tension

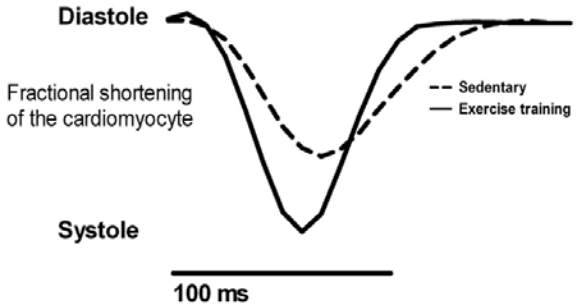

Figure 2. Computed traces of cardiomyocyte fractional shortening after electrical stimulation. Note the larger amplitude and faster time-course of the shortening in cardiomyocytes isolated from exercise trained hearts, compared to sedentary control hearts.

generation ability has also been shown in papillary muscles from exercise trained hearts (61). At the level of the cardiomyocyte, we (36, 37, 41, 56, 57) and others (35, 43, 62, 63) have in a series of experiments using rat and mouse models demonstrated that regular aerobic exercise training improves the maximal amplitude of the shortening in unloaded cells during electrical field stimulations; for illustration, see Figure 2. After a program of high intensity aerobic exercise at 85-90% of $\dot{V}O_{2max}$, cell fractional shortening increases 40-50%, whereas contraction-relaxation rates improve 20-40%. The exercise training-induced improvement is particularly consistent for relaxation rates; more so at high stimulation frequencies than low, suggesting that frequency-dependent acceleration of relaxation might also be affected by exercise training (36, 37, 56, 57). Moreover, we have also observed a faster contraction rate (56, 57). In line with this, faster shortening velocity has also been observed in cardiomyocytes under loaded contractions (62). In this study, force-velocity curves were also recalculated to indicate power output of the contracting cardiomyocyte, as power output is the product of force and velocity. This approach showed that maximal power output in the cardiomyocyte increased by ~60% after exercise training.

The magnitude of improvement is larger after high than moderate (65-70% of $\dot{V}O_{2max}$) exercise intensity; high intensity bringing about roughly twice the adaptation that moderate exercise enables (56). So far, this is the only report that has directly compared the extent of adaptation with different exercise intensities; with the caveat that the amount of exercise was not equalized. Nonetheless, this is in line with the observation that well-controlled studies at high aerobic intensity of the exercise regimen induce a greater adaptation than programs with lower exercise intensities.

Improved contractility develops steadily during the course of the exercise training program, until a plateau is reached after ~2 months (57). Hence, the positive inotropic effect observed after 8 weeks of regular exercise training was not different from that after 13 weeks. This is probably due to either the cardiomyocytes reaching a maximal potential for improvement, or that the relative intensity or the volume of the exercise need to be increased to exceed what it takes of exercise at this point to maintain the adaptation that has occurred. A few studies have been unable to find any effect of exercise training on cardiomyocyte contractility, or have reported a decrease in contractile function (64-67). Different exercise training protocols and experimental conditions, e.g. cell isolation procedures, ionic concentrations, pH, temperature, stimulation frequencies, and region of the heart from which cells were isolated from, may explain some of the variation between different studies. In our studies, we find that the exercise-induced effects are most marked when the cardiomyocytes are stimulated up to physiological frequencies (7-10 Hz; 36, 37, 56, 57), whereas most studies, including those that did not report beneficial effects of exercise, have not stimulated cells up to these frequencies (e.g. 64). Different experimental procedures may also partially explain the controversy regarding a positive or negative force-frequency relationship (68, 69). In our experimental conditions (physiological ionic concentrations, temperature, pH and stimulation frequencies), we typically observe a positive staircase during 2-7 Hz stimulation, but negative above 7 Hz (36, 37, 56, 57).

A greater extent and faster rates of shortening are not the only adaptations to regular exercise training. Experiments with permeabilized cardiomyocytes mounted between a motor positioner and a force transducer have demonstrated that force- and power production at the level of the single cell also increases (62, 70). Furthermore, exercise training increases the steepness of the active tension-sarcomere length relationship, suggesting that Frank-Starling mechanisms are altered also at the level of the single cardiomyocyte (70, 71). Thus, together with cellular hypertrophy (e.g. 35-37, 56-58); improved contractile capacity of the cardiac muscle cell forms a cellular basis for improved systolic output and diastolic filling, and hence a greater myocardial capability to perform external work. From whole heart and papillary muscle preparations, it has been shown that exercise training improves contractile function in the absence of changes in end-diastolic wall stress (11), and increases isometric force development when optimal sarcomere length is maintained (61, 72, 73). This suggests that contractile improvement with exercise training to a certain degree is independent of cardiomyocyte hypertrophy, and that the characteristics of myofilament crossbridge cycling and ATP hydrolysis are important.

4.1. Cardiomyocyte contractility and detraining

In contrast to exercise-induced adaptation in cardiomyocyte contractility, only one study has so far investigated the effects of ceasing an established, chronic exercise training program (detraining; 57). This study exercised rats at a high intensity (85-90% of $\dot{V}O_{2max}$) for 10 weeks, and reported substantial adaptation on cardiomyocyte fractional shortening and rates of contraction and relaxation, in line with the abovementioned discussion. Detraining, on the other hand, caused a rapid deterioration of the fractional shortening and rates of contraction-relaxation, as most of the training-induced

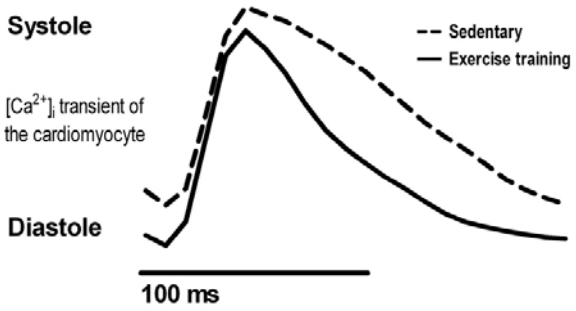

Figure 3. Computed traces of cardiomyocyte $[Ca^{2+}]_i$ transients after electrical stimulation. Note the lower diastolic $[Ca^{2+}]_i$ and the faster decay of the $[Ca^{2+}]_i$ transient in cardiomyocytes isolated from exercise trained hearts.

effects disappeared within 2 weeks and completely within 4 weeks of detraining. Thus, contractile improvement gained over 2.5 months with rigorous aerobic exercise vanished completely within only a month of detraining. In this study, detraining consisted of returning the rats to the same habitual sedentary lifestyle as before the exercise training program; it was not extended to immobilizing the animals, which would probably have accentuated the effects of detraining.

5. CARDIOMYOCYTE CALCIUM CYCLING AND EXERCISE TRAINING

The inter-dependency and the similar changes on $[Ca^{2+}]_i$ transients and contraction-relaxation velocities suggest that increase in rate of Ca^{2+} cycling explains the faster contraction-relaxation rates of the exercise-trained cardiomyocyte (see Figure 3; 37, 56, 57). In contrast, a larger fractional shortening after exercise training does not seem to be explained by elevated peak systolic $[Ca^{2+}]_i$, i.e. greater Ca^{2+} availability for the myofilaments (36, 37, 56, 57, 64). In fact, some studies have reported that both diastolic and systolic $[Ca^{2+}]_i$ are reduced after a program of exercise training (35-37), although this is not consistent with other studies that report no changes in either diastolic or systolic $[Ca^{2+}]_i$ (56, 57, 64, 74).

As discussed above, high aerobic intensity during exercise training appears to be more effective than moderate intensity for inducing contractile adaptation to the cardiomyocyte. The same study reported that the faster contraction-relaxation rates were associated with correspondingly faster rise and decay rates of the $[Ca^{2+}]_i$ transients in the same cells (56). In contrast, diastolic and systolic $[Ca^{2+}]_i$ were not different between different exercise intensities. Moreover, the regression of contraction-relaxation rates during a period of detraining does also correspond closely with regressed $[Ca^{2+}]_i$ transient rates, in terms of magnitude and time-scale of changes (57).

5.1. Mechanisms of altered cardiomyocyte calcium cycling after exercise training

Molecular changes that mechanistically explain the observed changes in Ca^{2+} handling have yet to be determined, although clues are provided. Prolonged action potential duration in exercise trained cardiomyocytes has been observed by several groups (70, 73), although it has also been suggested that a prolongation in the action potential only occurs in cells isolated from the sub-epicardium (70). As the voltage-dependent L-type Ca^{2+} channel is activated during the plateau phase of the action potential and the NCX operating in reverse mode during depolarization (21), a prolongation may lead to a greater Ca^{2+} entry across the plasma membrane. More effective coupling of the L-type Ca^{2+} current to RyR2 Ca^{2+} release, but not more channels, has also been shown with regular exercise (66, 75). This would suggest a mechanism for why exercise training might induce a higher rate of the rise of the systolic $[Ca^{2+}]_i$ transient (56). Exercise training-induced effects on isolated SR Ca^{2+} release *per se* have not been studied, but could potentially be interesting as the RyR2 complex is responsive to different stimuli (75-78). Faster $[Ca^{2+}]_i$ transient decay after exercise training seems to at least partly be explained by increased levels of SERCA2a and NCX (36, 37, 79-82), although other studies do not support this (83-85). The reason for this discrepancy is presently unclear, but may be due to different exercise intensities; studies of high exercise intensity report upregulated expression, whereas those of low exercise intensity do not report changes. Increased SERCA2a and NCX expression levels would improve Ca^{2+} re-sequestering and myocyte relaxation, and may secondarily improve contraction due to improved loading of the SR. However, one may also hypothesize that ion affinity, and hence activation pattern, and/or maximal activity of the cytosolic Ca^{2+} removers (SERCA2a and NCX) may change with regular exercise training, especially as long as the potential regulatory roles of the various protein kinase systems for adaptation to exercise training remain undetermined. Somewhat unexpectedly, it was also reported that PLN expression increases in the heart after exercise training (37). This would have the potential to neutralize the effects of increased expression of SERCA2a, if the ratio between phosphorylated and dephosphorylated PLB would remain intact.

The observed reduction of peak systolic $[Ca^{2+}]_i$ after exercise training (36, 37) could be due to several factors, such as reduced Ca^{2+} release into the cytosol, dilution of released Ca^{2+} into the cytosol due to cell hypertrophy, and improved Ca^{2+} buffering capacity inside the cell. The first two options are unlikely as they would suggest a reduced binding of Ca^{2+} to the myofilaments and hence reduced contractility; opposite to what is normally observed after exercise training (see above). The third option is feasible since only a small fraction of the released Ca^{2+} in the cytosol exists as free Ca^{2+} (86). This would also be consistent with reduced diastolic $[Ca^{2+}]_i$, also reported after exercise training (36, 37), and the observations of increased Ca^{2+} binding and binding sites in the exercise trained cardiomyocyte SR (87) and sarcolemma (82), respectively. Exercise training-induced adaptation of the sarcolemmal ATPase (88) and mitochondrial Ca^{2+} cycling (89) have also been suggested as mechanisms of lowered $[Ca^{2+}]_i$.

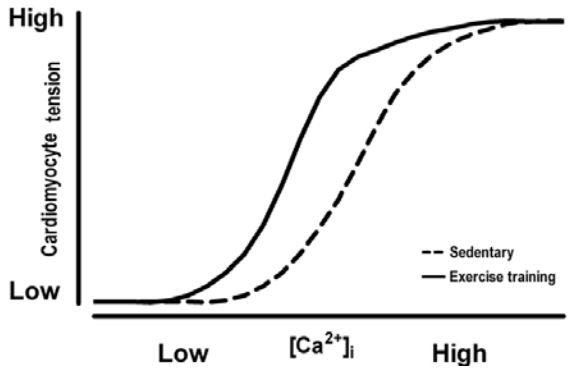

Figure 4. Computed traces of myofilament sensitivity to Ca^{2+} in cardiomyocytes. Note the lower $[Ca^{2+}]_i$ required to activate tension and induce half-maximum tension in cardiomyocytes isolated from exercise trained hearts, compared to sedentary control hearts. Maximal tension generation is not different between exercise trained and sedentary cardiomyocytes.

5.2. Improved calcium sensitivity accounts for greater fractional shortening

Since improved cardiomyocyte fractional shortening with exercise training does not appear to be explained by systolic or diastolic levels of $[Ca^{2+}]_i$ or transient amplitude (see above), it suggests that a greater magnitude of cell shortening results from improved myofilament responsiveness to Ca^{2+} (see Figure 4). Indeed, adaptation of cardiac Ca^{2+} sensitivity during submaximal, but not maximal activation of tension, to exercise training has been demonstrated thoroughly in rats (63) and is therefore suggested as a major reason for the changes in cell contractile capacity observed with exercise training. In this study, exercise training had no effect on maximal tension of cardiomyocytes, but produced a leftward shift in the tension-pCa^{2+} relationship. This has important consequences because almost all of the cardiac contraction occurs at submaximal $[Ca^{2+}]_i$. Hence, it suggests an improved activation with greater force output in the myocardium on a beat-to-beat basis. It has also been indicated that Ca^{2+} sensitivity improves with regular exercise training according to exercise intensity, and wanes with detraining (56, 57). With a different experimental approach, we have also measured Ca^{2+} sensitivity by studying cardiomyocyte length in passive, permeabilized cells subjected to increasing $[Ca^{2+}]_i$, and reported a greater contraction and hence an increased Ca^{2+} sensitivity after regular exercise training (37). Interestingly, this study also showed that Ca^{2+} sensitivity may be linked to pH_i; acidic conditions decreasing and alkaline conditions increasing cardiomyocyte Ca^{2+} sensitivity. Since protons may compete with Ca^{2+} and inhibit Ca^{2+}-binding to troponin C (47), it is feasible that pH_i regulation would affect myofilament sensitivity to Ca^{2+}. Indeed, exercise-induced changes in pH regulatory mechanisms have been suggested by increased mRNA expression levels of the Na^+/H^+ exchanger (NHE) after a program of exercise training (37). Other mechanisms of altered Ca^{2+} sensitivity might be found in the contractile machinery of the cell. Both gene and protein levels of atrial myosin light chain-1 in the heart are increased with exercise training, as confirmed by microarray hybridizations, polymerase chain reactions and two-dimensional gel electrophoresis (43), but the exact physiological role needs be clarified. A potential role may be linked to the N-terminal end of the atrial myosin light chain-1, which affects binding to actin (90). Also, isoform shifting of troponin T (42), troponin I (44), and myosin heavy chains (45) has been associated with altered Ca^{2+} sensitivity, although changes in response to exercise training have not been studied yet.

Length-dependence of sarcomere Ca^{2+} sensitivity; stretched sarcomeres producing more force, implies that the Frank-Starling relationship between end-diastolic filling and stroke volumes is also linked to myofilament Ca^{2+} responsiveness. Exercise training affected Ca^{2+} sensitivity such that a change in cell or sarcomere length (from 1.9 to 2.3 μm) had a larger effect in Ca^{2+} sensitivity of tension in exercise trained, as opposed to sedentary control cardiomyocytes (71). Thus, exercise training imposes an increase in the length-dependence of Ca^{2+} sensitivity, which is translated to a greater tension production at any given sarcomere length. This length-dependence of Ca^{2+} sensitivity may be the reason for why a lack of exercise training effect on Ca^{2+} sensitivity also has been reported (70), as this study investigated cardiomyocytes at short sarcomere lengths. However, since the variation on Ca^{2+} sensitivity between different species is considerable (25, 27, 39, 48-50) and no exercise training data beyond those from rats exist, the possibility remains that improved Ca^{2+} sensitivity is species-specific.

6. CARDIOMYOCYTE CONTRACTILITY AND CALCIUM CYCLING IN HEART FAILURE

Severe heart failure is characterized by progressive global myocardial dysfunction and ventricular dilatation, which increases morbidity and mortality. In the cardiomyocytes studied from various heart failure models, there is typically an abnormal excitation-contraction coupling, a reduction in fractional shortening and hampered Ca^{2+} homeostasis, normally coinciding with downregulated SR Ca^{2+} cycling and upregulated NCX function that tend to shift Ca^{2+} out of the cell and reduce SR Ca^{2+} content. This contributes to the $[Ca^{2+}]_i$ transients usually being smaller and slower, but with higher diastolic $[Ca^{2+}]$. Together with leaky RyR2s, these changes contribute to a systolic and diastolic dysfunction that reduces the ability to perform work and impairs diastolic ventricular filling. As of yet, it is not clear which role Ca^{2+} sensitivity of the myofilaments play as both up- and downregulation has been shown. It has been suggested that Ca^{2+} sensitivity may take a compensatory role in volume overload hypertrophy because of the stretching of the sarcomeres (see above and 39). Major research efforts worldwide are focusing on understanding myocardial biology in heart failure, hypertrophy and dysfunction, including investigations of the potential effects of various clinical and experimental treatment options, such as pharmacological interventions to unload the heart or alter excitation-contraction coupling, contractile function or cardiac metabolism, and onto the applicability of gene therapy and stem cells to regenerate myocardial tissue. It would be beyond the scope of this

review to go into detailed mechanisms of cardiac dysfunction and abnormal contractile function and Ca^{2+} cycling, but the interested reader is referred to a number of excellent reviews, e.g. (21, 22).

Another strategy gaining momentum for restoring myocardial dysfunction is the use of regular exercise training. Clinical studies have already demonstrated that regular exercise training is beneficial in cardiovascular disease patients and in those at risk of developing disease (91-94), and that the level of aerobic fitness predicts survival in healthy as well as in a cardiovascular disease population, even when other traditional risk factors are present or in the setting of established β-blockade (95-97). Potential beneficial effects of exercise training in heart failure that directly impact on the myocardium are restoration of metabolic function (98), and regressed remodeling (36). It is also possible that exercise training modulates myocardial pump function at the level of the single cardiomyocyte.

7. EXERCISE TRAINING IMPROVES FAILING MYOCARDIAL FUNCTION IN HEART FAILURE

7.1. Exercise training and contractility in heart failure

In rats, exercise training at a high relative aerobic intensity (85-90% of $\dot{V}O_{2max}$) restores fractional shortening in cardiomyocytes from heart failure rats, in which fractional shortening is clearly depressed when the animals remain sedentary (36). The extent of the improved contractility was such that it after regular exercise training in heart failure reached the levels of sedentary sham-operated healthy controls. These results were demonstrated in freshly isolated cells from the viable myocardium, studied at 37°C and stimulated up to physiological heart rates (up to 10 Hz). Both fractional shortening and the rate of cardiomyocyte relengthening improved with exercise training. Similar effects were reported after a program of anaerobic short-bout sprint training starting 3 weeks after myocardial infarction (99), despite this exercise intensity being well above the current recommendations in cardiology clinics. Nonetheless, both programs normalized the amplitude of the cell shortening in failing cardiomyocytes, and restored contraction and relengthening rates to velocities comparable to normal, healthy cells.

The abovementioned studies indicate that exercise training may assert an important direct effect on myocardial biology in heart failure, and provides a cellular rationale for investigating physical activity and exercise training in heart failure patients. The magnitude of the exercise training effects observed in heart failure cardiomyocytes were comparable to the effects in cardiomyocytes isolated from healthy, normal hearts without any dysfunction. Thus, although myocardial contractile function is depressed in heart failure, the ability to adapt to regular exercise training seems to be intact.

7.2. Exercise training and calcium cycling in heart failure

In line with improved contractility in failing cardiomyocytes after aerobic exercise training, $[Ca^{2+}]_i$ homeostasis and cycling also improve towards healthy levels, and hence provides a mechanism for the restored contractility. Exercise training reduced the elevated diastolic $[Ca^{2+}]$, and restored the amplitude and the rates of the rise and decay of the $[Ca^{2+}]_i$ transients to normal levels (36). In contrast to most studies of failing cardiomyocytes, this study also reported raised systolic $[Ca^{2+}]_i$ as compared to sham-operated control cells. The reason for this somewhat unexpected observation was not clear, but similar results have been observed in sub-endocardial cells (100). Nonetheless, this systolic increase was also shifted towards normal levels after exercise training. However, despite exercise training reduced diastolic (and systolic) $[Ca^{2+}]_i$, it did not completely restore to normal levels, as there still was a 5-10% difference between healthy and heart failure cardiomyocytes (36). A likely mechanism for alleviating impaired Ca^{2+} handling with exercise training in heart failure was upregulated SERCA2a expression in the heart, suggesting improved SR Ca^{2+} cycling. NCX expression levels were also regulated towards normal levels, but in this particular model, heart failure was not associated with increased NCX expression, but instead with reduced expression levels. Nonetheless, the reported changes with exercise training would have shifted Ca^{2+} homeostasis and cycling towards normal levels; both SERCA2a and NCX expressions reached the levels of healthy hearts after exercise training in heart failure. As reported above (99), anaerobic sprint training caused similar improvements to cardiomyocyte contractility as aerobic exercise training. Anaerobic exercise training also restored $[Ca^{2+}]_i$ transient amplitude and rates of rise and decay (74, 101), but in contrast to aerobic exercise training, anaerobic sprint training caused a further reduction in SERCA2a and PLN expression levels. Also, in this model, systolic $[Ca^{2+}]_i$ was reduced in heart failure, whereas anaerobic sprint training brought about an increase, but which in this case was a restoration of systolic $[Ca^{2+}]_i$. Furthermore, a pathologically long action potential duration was shortened with anaerobic sprint training, possibly by enhancing the fast and slow components of the transient outward Ca^{2+} current in the failing cardiomyocytes, albeit not completely to sham-operated levels (102). In mice, it was recently shown that exercise training starting early after myocardial infarction (1 day after) did not alter $[Ca^{2+}]_i$ transients, but that diastolic $[Ca^{2+}]_i$ was reduced to normal levels (103).

Collectively, these observations show that exercise training improves failing contractile function at least partly by improving Ca^{2+} dynamics in surviving cardiomyocytes, although the actions of different exercise training programs on $[Ca^{2+}]_i$ transients may differ. Exercise training also tends to restore impaired contractility and Ca^{2+} handling in a rat model of metabolic syndrome with depressed myocardial function, as recently reported (41).

7.3. Exercise training and calcium sensitivity in heart failure

Peak systolic $[Ca^{2+}]_i$ does not explain the larger extent of the cardiomyocyte shortening observed after regular exercise training in healthy rats. How this is in cardiomyocytes isolated from heart failure rats is less

Table 1. Summary of cardiomyocyte contractility and Ca^{2+} cycling adaptation to regular exercise training, to heart failure, and to exercise training in heart failure

	Exercise training	Heart failure	Exercise training in heart failure
Amplitude of shortening	↑	↓	↑
Rates of contraction-relaxation	↑	↓	↑
Force-length relationship	↑	↓	?
Power output	↑	↓	?
Peak systolic $[Ca^{2+}]_i$	-↓	-↑	↓
Diastolic $[Ca^{2+}]_i$	↓	↑	↓
$[Ca^{2+}]_i$ transient amplitude	-↓	↓	↑
Rates of rise and decay of $[Ca^{2+}]_i$ transient	↑	↓	↑
Myofilament Ca^{2+} sensitivity	↑	↕	↕ (1)

Arrows denote up- or downregulation and flat line denotes no regulation. $[Ca^{2+}]_i$: intracellular Ca^{2+} concentration. ?: No studies of this aspect exist. (1): two reports of myofilament Ca^{2+} sensitivity in cardiomyocytes isolated from exercise trained heart failure animals exist; in these studies, heart failure was associated with both up- and downregulated Ca^{2+} sensitivity, which exercise training corrected in both instances (see text for details).

known, especially as it has not yet been settled how Ca^{2+} sensitivity develops in failing cardiomyocytes (see discussion above). Two studies have examined Ca^{2+} sensitivity after aerobic exercise training in heart failure, one in rats and one in mice. In rats, it was found that depressed Ca^{2+} sensitivity in post-myocardial infarction heart failure was normalized after a program of aerobic treadmill running (36). This would be comparable to the action of Ca^{2+} sensitizers that improve contractile function in heart failure (51) and hence supports important clinical effects. It was also noted that failing cardiomyocytes isolated from exercise trained rats were less affected by experimental changes in pH, compared to failing cardiomyocytes from sedentary rats, suggesting a similar link between pH_i regulation and Ca^{2+} sensitivity as in exercise trained healthy individuals (36). However, other studies using similar models have reported that Ca^{2+} sensitivity is increased, not reduced, in heart failure, including the second study of Ca^{2+} sensitivity after exercise training in heart failure (103). Ca^{2+} sensitivity was increased in mice post-myocardial infarction, which exercise training was able to restore to normal levels. Possible mechanisms noted were increased cAMP levels suggesting PKA and β-adrenergic dependence, and increased phosphorylation of myosin light chain 2, whereas myosin heavy chain composition was unchanged. However, heart failure subsequent to myocardial infarction was not observed in this study. Hence, the understanding of myofilament Ca^{2+} sensitivity after exercise training in heart failure remains incomplete.

8. GENERAL CONSIDERATIONS AND CONCLUSIONS

In summary, it has been demonstrated that a long-term program of exercise training improves the contractile capacity of the cardiomyocyte, and that this effect mainly is attributed to improved Ca^{2+} cycling and homeostasis. Major effects are brought about by faster cycling of Ca^{2+}; especially in diastole, and a greater sensitivity of the myofilaments to given $[Ca^{2+}]_i$. These effects provide cellular mechanisms for improved pump capacity and a greater ability to perform external work of the exercise trained heart. It also implies that the cardiomyocyte is highly adaptive to exercise training, and provides a cellular mechanism for changes in overall exercise capacity and $\dot{V}O_{2max}$. Beneficial effects of exercise training have been demonstrated in both unloaded and loaded cardiomyocytes, with different experimental approaches, and in models of both normal and compromised cardiac function. An interesting correlative here is how exercise training compares to conventional pharmacological treatment for failing cardiomyocyte contractile function and Ca^{2+} cycling. It has been reported that exercise training had beneficial effects comparable to angiotensin II type 1 treatment with Losartan that aims to reduce the pathologically high blood pressure and hence unload the heart; both exercise training and Losartan nearly restored the diminished fractional shortening and Ca^{2+} cycling and the contraction-relaxation rates thereof to a similar extent (36,38). For summary of exercise training-induced effects on cardiomyocyte contractility and Ca^{2+} cycling in healthy and in heart failure, see Table 1.

Despite the potential exercise training has to combat myocardial dysfunction and heart failure, there are conditions where exercise training may be contraindicated, such as various forms of valvular heart disease. For instance, whereas patients with mild to moderate aortic stenosis seem to tolerate physical activity and exercise training well, this is not recommended in severe aortic stenosis, due to the elevated risk of cardiac arrest and effort syncope (104). Moreover, it has recently been established that a mutation in the RyR2 gene reduces the affinity of the stabilizing protein FKBP12.6 to RyR2, leading to diastolic Ca^{2+} leak that causes exercise-induced sudden cardiac deaths (105).

The above discussion is based upon experimental results from studies of small animals. This has two major implications; species differences and the nature of the experimental exercise programs. Firstly, given that excitation contraction coupling, Ca^{2+} cycling and contractile function is by far similar between different species, including man; the main differences are quantitative rather than qualitative, we believe that with proper caution, these results are also applicable to man. Importantly, as far as clinical studies have been able to

study, there is a strong correlation between exercise training-induced effects in small animals and men. On the lower end of the "dysfunction-to-athleticism" spectrum, where studies of human cardiomyocytes have been possible when isolated from transplanted and diseased hearts; a close similarity between small animals and men has been revealed (24, 25). Also, it has been demonstrated that cardiomyocyte morphology and function is fairly similar between animals that are smaller than humans and those that are larger, i.e. mouse to horse (106). Secondly, experimental exercise programs in small animals are somewhat different than in humans, particularly on compliance, confounding factors and regularity of exercise training sessions. Hence, the magnitude of improvements may not be directly transferable to clinical situations. Finally, sex, age, different genetic backgrounds, and the design of the exercise program may influence the outcome.

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Abbreviations: $[Ca^{2+}]_i$: intracellular Ca^{2+} concentration, DHPR, dihydropyridine receptor (L-type Ca^{2+} channel), NCX: Na^+/Ca^{2+} exchanger, NHE: Na^+/H^+ exchanger, pH_i: intracellular pH, PLN: phospholamban, RyR2: ryanodine receptor-2, SERCA2a: sarcoplasmic reticulum Ca^{2+} ATPase-2a, SR: sarcoplasmic reticulum, $\dot{V}O_2$: oxygen uptake, $\dot{V}O_{2max}$: maximal oxygen uptake

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