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Exercise and calcium in the heart

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Cardiomyocyte Ca^{2+} dictates cardiac contraction via excitation–contraction coupling (ECC) and excitation–transcription coupling. Adaptation to these processes also majorly contributes to enhanced contractile function and capacity following exercise training. Cytoplasmic Ca^{2+} release controls sarcomeric contraction, with important modulation by the voltage-sensitive plasma membrane L-type Ca^{2+} channel and the Ryanodine receptor, as well as the sarcoplasmic reticulum Ca^{2+} ATPase. Exercise training increases and enhances these ECC subprocesses, in a manner that increases and enhances cardiac contraction. Also, adaptation to exercise training further includes myofilament Ca^{2+} sensitization. Then, there are several aspects linked to postexercise training cardiomyocyte Ca^{2+} handling that remains speculative and inconclusive, but could if proven true to be of special importance. This includes Ca^{2+}-linked muscle-specific gene transcription to alter cell architecture and size, and it includes the scenario whereby Ca^{2+} cycling and adaptations may alter arrhythmogenicity. These aspects of cardiac Ca^{2+} adaptations to exercise training are discussed in this review article.

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Introduction

The heart plays a major role in determining a person's physical and exercise capacity via its pumping of blood around the body: the greater the pump capacity, the greater the exercise capacity, at least from a physiologic point of view. Not all aspects of this have been fully understood yet and there is still debate surrounding this organ, but most will however agree that the heart plays a crucial role in setting the exercise capacity as well as underlying the adaptation that allows people to increase exercise capacity following programs of physical activity and exercise training [1,2]. For clarity, exercise capacity and exercise training are reoccurring terms in this review, where exercise capacity refers to the physical and physiologic ability a person has to perform exercise, which functionally may be graded from light to maximal and it is the maximal ability that often is in question, while exercise training refers to a long-term program of frequent exercise sessions a person may undertake, usually with the aim of improving exercise capacity. Hence, exercise capacity is something everyone 'has,' and a fit person usually has a greater exercise capacity than an unfit, whereas exercise training is something someone does, though not everyone (perhaps unwisely) will choose to do.

Ca^{2+} initiates and modulates several important processes in the heart and the heart muscle cell (cardiomyocyte) that govern the heart's role in exercise, including control of the heartbeat itself as well as the size of the heart's myocardium and its responses (adaptations) to exercise training. In fact, the very Ca^{2+} that controls the heartbeat may also simultaneously modulate heart size, albeit the latter takes a long time, and vice versa. Finally, and perhaps a discovery of late, Ca^{2+} may also turn ugly, as there now are well-described scenarios where it may cause arrhythmias and sudden death, or oppositely, save one from them. This review covers the role of Ca^{2+} in the cardiomyocyte as the cell and the heart adopts to exercise training, with a focus on the latest developments.

Ca^{2+}

The cardiomyocyte is the principal cell in the heart, at least for contractile and pump purposes. Here, Ca^{2+} acts as a cellular messenger that controls a number of processes, including intrinsic sarcomeric and myofilament contraction–relaxation and tension development; it carries charge and regulates voltage, and is involved in gene transcription, cellular remodeling, and apoptosis. Ca^{2+} is ubiquitous to cells, but inside the cell, it sequesters into several compartment organelles such as the sarco-/endoplasmic reticuli, mitochondria, nuclei, or it exists in either free or buffered forms in both extra- and intracellular spaces [3]. Because of this compartmentalization, Ca^{2+} concentration ($[Ca^{2+}]$) gradients are created,

Nomenclature

AP	Action potential
MEF	Myocyte enhancer factor
Ca^{2+}	Calcium
mRNA	messenger Ribonucleid acid
$[Ca^{2+]}$	Calcium concentration
NCX	Sodium/Ca^{2+} exchanger
CaMK	Ca^{2+-}calmodulin dependent protein kinase
NFAT	Nuclear factor of activated T-cells
CICR	Ca^{2+}-induced Ca^{2+}-release
PLB	Phospholamban
DNA	Deoxyribonucleic acid
PMCA	Plasma membrane Ca^{2+} ATPase
ECC	Excitation-contraction coupling
RyR	Ryanodine receptor
HDAC	Histone deacetylace
SERCA	Sarcoplasmic reticulum Ca^{2+} ATPase
IP3R	Inositol triphosphate receptor
SR	Sarcoplasmic reticulum
LTCC	L-type Ca^{2+} channel
VO_{2max}	Maximal oxygen uptake

which as a result create specific spatial and temporal fluxes and targeted signals inside the cell by release, uptake, or transfer via ion-selective and -specific channels, pumps, and exchangers across cell or organelle membranes [4]. Important examples here include the space between the sarcoplasm, the sarcoplasmic reticulum (SR), and the sarcomeric myofilaments, with Ca^{2+} being released from and removed to extracellular spaces and the lumen of the SR, while the space consisting of the nucleus, nuclear envelope, and endoplasmic reticulum provides another example. In these two intracellular loci, Ca^{2+} exists in different concentrations and signals for different cellular events.

The heart is a pump driven by Ca^{2+}

Cardiomyocyte excitation–contraction coupling (ECC) is the basis for the heartbeat, including its rhythmicity and strength. It initiates, controls, and coordinates a uniform myocardial systolic contraction and diastolic relaxation and largely influences the active tension developed by the myocardium during the contraction. The intracellular events are initiated by the action potential (AP) depolarizing the plasma membrane, which due to its speedy millisecond propagation throughout the whole myocardium, results in individual cardiomyocytes contracting near-synchronously in a pattern set by the direction of the AP propagation from the apex to the base, and in a highly rhythmic pattern due to the properties of the sinoatrial node, and all with a high fidelity [5]. In the individual cardiomyocyte plasma membrane, the depolarization initiates an inward Ca^{2+} current through the L-type Ca^{2+} channel (LTCC) that thereby increases the $[Ca^{2+}]$ in the dyadic space between the plasma membrane or its inward-protruding extension, the transverse tubule, and the SR. Dyadic Ca^{2+} stimulates Ryanodine receptor (RyR) on the SR membrane to open, which then releases stored bulk Ca^{2+} sufficient to increase cytoplasmic intracellular $[Ca^{2+}]$ from ~0.1 μM to 0.5–1 μM; a process also known as Ca^{2+}-induced Ca^{2+} release (CICR). Cytoplasmic Ca^{2+} binds to and conformationally shifts the regulatory troponin–tropomyosin complex of the myofilaments to a position that allows actin–myosin interaction, and this generates the cross-bridge cycling that results in sarcomeric and therefore cell shortening and thereby contraction in systole. This is immediately followed by intracellular free and troponin-bound Ca^{2+} being resequestered back to the Sarcoplasmic reticulum by the Ca^{2+} ATPase (SERCA) or transported out of the cell by the Na^{+}/Ca^{2+} exchanger (NCX) and the plasma membrane Ca^{2+} ATPase (adenosine triphosphatase) (PMCA), which thereby reduces intracellular Ca^{2+}, brings it back to baseline levels, and therefore allows for sarcomeric and cell relengthening and relaxation in diastole [5,6].

Activator Ca^{2+}

The role of activator Ca^{2+} has been investigated in a series of experimental studies of chronic exercise training. Exercise training has mainly been achieved by voluntary free-wheel or controlled treadmill running or swim-training typically in mice or rats; the cell specimen has mainly been that of freshly isolated single cells that are bathed in standardized and temperature-controlled perfusion chambers and that are subject to electrical field twitch stimulations at different frequencies that mimic physiological heart rates, while animals have been sacrificed either with or without (control) exercise training backgrounds. The Ca^{2+} signal is achieved by epifluorescence or laser confocal excitation and scanning of Ca^{2+}- sensitive fluorescent indicators, for example, Fluo- or Fura-based probes that are experimentally delivered into the cell or specific compartments inside the cell [7].

Since the contraction–relaxation process and contractility are largely determined by ECC and intracellular Ca^{2+} handling, it follows that if exercise training is to improve cardiac contractility and pump function, which any exercise physiology student would agree seems to happen [8], then we should also expect to see exercise training-induced improvements in ECC and the Ca^{2+} handling process; however, although this more often than not has been shown to be true, it has not always been the case, and Ca^{2+} effects have as such not been quite as convincing as contractile effects following exercise training, even though in a number of different experimental and

clinical scenarios, an intimate link between cardiomyocyte transmembrane and intracellular Ca^{2+} handling and contractility has been demonstrated [9]. Nonetheless, on the specifics of exercise training-induced adaptations to cardiomyocyte Ca^{2+} handling, we find:

L-type Ca^{2+} channel and Ca^{2+}-induced Ca^{2+} release (with focus on *Ca^{2+}-induced* in *Ca^{2+}-induced* Ca^{2+} release)

Despite LTCC and its associated plasma membrane, Ca^{2+} influx is of major importance for ECC and intracellular release of Ca^{2+}, this is the aspect of cellular Ca^{2+} handling that has received the least amount of scientific attention with respect to exercise training adaptations. Nonetheless, an indirect suggestion indicating it might be important came with the finding that the ventricular AP was prolonged as a result of exercise training, at least in specific layers of the myocardium [10], as the implication was that the longer depolarization may potentially prolong the activation time of LTCC, because it is voltage-sensitive [11]. This would enhance the inward Ca^{2+} influx. Such an effect would then potentially also increase CICR, since RyR activation is Ca^{2+}-sensitive. However, direct evidence of this was not forthcoming until a report in 2016 showed that wild-type mice performing daily high-intensity exercise training for 6 weeks increased both the LTCC Ca^{2+} current flux and density by ~80% [12], as measured by the single-cell voltage-clamp technique. This effect was not linked to protein expression of LTCC and was instead thought to involve increased activation of the Ca^{2+} channels already present in the plasma membrane before exercise training commenced, which would be in agreement with the previous finding of AP prolongation following exercise training [10]. Intriguingly though, LTCC expression did substantially increase in transgenic mice with chronically enhanced Ca^{2+}–calmodulin-dependent protein kinase (CaMK) activity [12], suggesting that if exercise training is capable of activating CaMK, it may then instigate signaling that increases LTCC expression. However, further reports have not been able to corroborate a possible LTCC or Ca^{2+} flux effect: first, 4 weeks of voluntary wheel running did not affect LTCC expression in the heart [13], but this volume and intensity of exercise training are considerably less than most other studies, including those reviewed above [10,12]; and second, 12 weeks of 1–2 h/day, 5 days/week swim-training in rats that did lead to robust cardiac and cardiomyocyte structural and functional adaptations, did also not induce any effects on LTCC gene (mRNA) or protein expression or LTCC influx properties, quantified by the current–voltage relationship, which as such means LTCC was assessed both biochemically and electrophysiologically [14]. The latter study in particular is robust in its execution, combines a number of experimental techniques, includes a long exercise training program (albeit without control of exercise intensity), and does report hallmark cardiac and cardiomyocyte changes that one would expect from a successful exercise training program, so it is not easy to reconcile the lack of Ca^{2+} channel effects in this study with the more positive findings reported earlier [10–12], although it remains possible that electrophysiologic adaptations are also at least partly dictated by exercise training mode and/or intensity and that differences here propel differences in study outcomes.

Ryanodine receptor and Ca^{2+}-induced Ca^{2+} release (with focus on *Ca^{2+} release* in Ca^{2+}-induced *Ca^{2+} release*)

The majority of RyRs are junctional SR-bound, existing in the tight dyadic space between SR and plasma/transverse tubule membranes, where they form functional clusters located within nanometer distance of LTCCs [15]. Since RyR opening is activated by Ca^{2+} binding to its cytoplasmic region, with high sensitivity, RyR opening therefore occurs rapidly in response to the LTCC influx or saltatory by the Ca^{2+} already released from nearby RyRs in a fire-diffuse–fire mode [15]. This favors rapid and uniform SR Ca^{2+} release as well as a rapid Ca^{2+} flux to adjacent sarcomeres, due to space restrictions in the dyad and the consequently fast Ca^{2+} signaling out of this space. Hence, an exercise training-induced increase in LTCC Ca^{2+} influx would also potentially affect RyR Ca^{2+} release. A second important consideration is that RyR Ca^{2+} release is also a function of the SR Ca^{2+} load [16]; the SR store determines the maximal release as the quantity of release cannot exceed the quantity stored, and SR Ca^{2+} load is primarily a function of SR Ca^{2+} uptake via SERCA [6]. Although specific RyR-gated Ca^{2+} flux before and after exercise training has not been quantified, the magnitude of the Ca^{2+} released during CICR, measured as the intracellular Ca^{2+} transient taken from [Ca^{2+}] measurements through cycles of Ca^{2+}-release events, provides an assessment of RyR release. This is because of the total Ca^{2+} release, the RyR contributes the majority Ca^{2+}, whereas release from LTCC, NCX, inositol-1,4,5-triphosphate receptors, and mitochondria contributes only minimally [17].

Early measurements of intracellular Ca^{2+}-transient amplitudes showed that exercise training did not increase RyR Ca^{2+} release [18–21]; however, rates of rise (and decay) of the Ca^{2+} transient increased [12,14,18–24]. Thus, even though the Ca^{2+} transient did not increase in magnitude, it was thought that the altered shape by the faster Ca^{2+} handling might functionally reduce 'smearing' and generate a 'sharper' Ca^{2+} transient peak with more ions reaching the contractile myofilaments at the same time, such that functionally myofilaments may be stimulated by a better-suited Ca^{2+} transient for evoking contraction, than the measurements might indicate, given they ascertain whole-cell intracellular Ca^{2+} and not smaller subcellular loci. Newer studies however started to report that exercise training also increased the size of the

Ca^{2+} transient and therefore the size of the RyR Ca^{2+} release [12,14,22–24], in a departure from the previous notion. This included the same laboratories that initially had not observed an increase. The reason for this discrepancy remains unknown, but it has by now become accepted that exercise training may also increase cardiomyocyte CICR and the magnitude of intracellular Ca^{2+} release, while the fact that exercise training increases rates of Ca^{2+} handling has been more or less a long-held notion. Mechanisms that explain the increased Ca^{2+} transient include the abovementioned increase in LTCC influx-activated stimulation of RyR, which constitutes a major factor in increasing RyR-induced Ca^{2+} release, but at least two other mechanisms have also been suggested that relate to intrinsic RyR Ca^{2+} release and SR loading, namely intrinsic RyR and SERCA expressions and functions: 1) RyR expression: a recent study found that swim-exercise training increased cardiomyocyte RyR gene (mRNA) expression 30–40% [14], while another study showed a small, albeit insignificant, trend toward increased RyR protein expression [13]. Together with exercise training also increasing gene expression for Calsequestrin [14], which is involved in luminal SR Ca^{2+} binding and increasing RyR gating and therefore open probability, it follows that increased RyR and Calsequestrin may also increase the amount of released Ca^{2+} from the SR during CICR. 2) SERCA expression and function: even though the primary role of SERCA has been seen as to remove and resequester cytoplasmic Ca^{2+} back to SR and thus remove activator Ca^{2+} in diastole, this function also recharges the SR with Ca^{2+} [6].

Sarcoplasmic reticulum Ca^{2+} ATPase

SERCA determines SR Ca^{2+} reuptake rate and content, and therefore also RyR Ca^{2+} release [6]. It has experimentally been well-documented that the size of the SR Ca^{2+} store in the cell drives not only caffeine-stimulated Ca^{2+} release, but also twitch-stimulated Ca^{2+} release and Ca^{2+} transient, which mimics normal mode of function [6,16]. Thus, exercise training-induced adaptations in SERCA will not only affect the rate of cytoplasmic Ca^{2+} removal and SR Ca^{2+} uptake, but in so doing also allow for an increase in intra-SR Ca^{2+} loading and content that consequently increase RyR Ca^{2+} release and CICR. Exercise training-induced shortening of intracellular Ca^{2+} rise and decay times, and especially decay times, all indicating faster rates, have been consistent across experiments [12,18–24] in rats and mice and across the range of applicable electrical stimulation frequencies that replicate measurements being taken at different heart rates; the adaptations have been sensitive to exercise training intensities, high-intensity interval training at 80–90% of maximal oxygen uptake (VO_{2max}), yielding greater changes than moderate-intensity training at 60–70% of VO_{2max} [18], and the effect has receded when exercise training has been withdrawn by detraining [19]. In the years since the original experiments, subsequent experiments have confirmed and reiterated the initial findings [14], and thus, this part of the intracellular Ca^{2+} handling narrative may be considered fairly robust: exercise training leads to faster Ca^{2+} handling. The faster rate of the intracellular Ca^{2+}-transient decay following exercise training has been explained by concomitant findings of increased gene and protein expressions of SERCA [13,21–25], which in and of itself alone may explain the faster Ca^{2+}-transient decay following exercise training, since SERCA solely resequesters the majority (70–90%) of the cytoplasmic Ca^{2+} after Ca^{2+}-release events [6]. However, and in addition, Phospholamban (PLB), an innate inhibitor of SERCA, does not seem to change expression levels with exercise training [23–25], such that SERCA-to-PLB ratio also changes in favor of enhanced SERCA activity after exercise training. Direct evidence of increased SERCA Ca^{2+} uptake following exercise training came from experiments in permeabilized cardiomyocytes that assayed the isolated SERCA Ca^{2+} uptake function across a full physiological range of cytoplasmic [Ca^{2+}], showing an increase of ~30% in exercise-trained cardiomyocytes versus controls [25]. Moreover, exercise training also induced hyperphosphorylation of CaMK, with consequent downstream hyperphosphorylation of Threonine-17 PLB [23]. This is physiologically important because PLB phosphorylation blocks PLB from suppressing SERCA [6], and this therefore also contributes to the enhanced lusitropic and inotropic effect of exercise training. Subsequently, another regulatory PLB site, Serine-16, has also been observed to increase after exercise training [22], with the same positive effect on intracellular Ca^{2+} handling and contractility.

Na^+/Ca^{2+} exchanger and diastolic Ca^{2+}

SERCA is the major mechanism by which the cardiomyocyte re-establishes low baseline levels of cytoplasmic [Ca^{2+}] after Ca^{2+}-release events (in other words, in cellular diastole) [6], and regulation of SERCA is consequently also the major pathway for exercise training-induced changes in cytoplasmic [Ca^{2+}] in diastole [25]. However, other cellular processes may also contribute, such as NCX, PMCA, mitochondria, and intracellular Ca^{2+} buffers [6]. Although some studies have suggested exercise training may rescue NCX gene and protein expression in situations where NCX is downregulated, such as after cardiac injury [20] or sympathetic hyperactivity [26], most studies in healthy hearts have not found any evidence of exercise training-induced adaptation [13,14,21,22]. However, one study [20] reported an increase in NCX protein expression and another study [27] indirectly indicated that Ca^{2+} efflux may increase after exercise training, suggesting exercise training may under some circumstances lead to an increase, for example, if conducted with high intensity or volume/duration. Simplistically, in order to balance Ca^{2+} influx with efflux on a beat-to-beat basis, and since

exercise training leads to AP prolongation and increased LTCC that consequently may increase Ca^{2+} influx, one might expect a concomitant increase in Ca^{2+} efflux, which questions why NCX appears not to adapt. However, since exercise training decreases diastolic [Ca^{2+}] [18–21,23], likely due to the SERCA effect [25], it transpires that the requirement to shift Ca^{2+} out of the cell may have diminished, and so no changes to NCX expression may in fact effectively represent an increased capacity to efflux Ca^{2+} in diastole. Moreover, it should also be noted that in circumstances with low SERCA expression and function, the NCX is also, at least up to a point, capable of extruding more Ca^{2+} than otherwise observed during normal cellular homeostasis [28], and finally, mitochondrial Ca^{2+} handling and intracellular Ca^{2+} buffering may also increase capacity following exercise training [29–31], and in such a way contribute to reduce diastolic [Ca^{2+}] and enhance Ca^{2+} handling.

Myofilament Ca^{2+} sensitivity

Increasing activator Ca^{2+} via CICR in the cardiomyocyte following exercise training in order to enhance contractile function, as detailed above, is one thing; increasing the sensitivity and responsiveness in the contractile machinery inside the cell to that Ca^{2+} is another. Changing the response with which myofilaments react to a given [Ca^{2+}] may modulate contractile function without the need to change Ca^{2+} release and handling during ECC. In elegant studies of Ca^{2+} sensitivity in permeabilized cardiomyocytes that allows for fine titration of intracellular [Ca^{2+}] and with the cell attached to microscopic force transducers to directly measure cellular tension, it was shown that exercise training increases myofilament Ca^{2+} sensitivity during submaximal tension activation, but not necessarily during maximal tension [32]. The increase during activation of submaximal tension is important, because this is where the bulk in vivo contraction occurs, with submaximal tension at submaximal intracellular [Ca^{2+}]. In other words, a leftward shift in the pCa-tension curve after exercise training was observed, meaning that contraction (or tension) was generated at lower intracellular [Ca^{2+}], or alternatively, a higher tension was generated at comparable [Ca^{2+}] in the exercise-trained cardiomyocyte versus the untrained control. For a long time, this was the only direct evidence of exercise training-induced sensitization of myofilaments to Ca^{2+}, although indirect evidence also supported this [18–21]. Very recently, similar permeabilized single-cell experiments to the original direct measurements confirmed yet again that treadmill running exercise training sensitizes the myofilaments to Ca^{2+} [33], and finally, similar effects were also observed in experiments of left ventricular papillary muscles following swim-training [34]. Thus, the available evidence shows that exercise training sensitizes or increases Ca^{2+} sensitivity of myofilaments. However, a full mechanism for this adaptation has yet to be identified, but several contenders have been suggested:

Intracellular potential of hydrogen

Maintenance of normal intracellular potential of hydrogen (pH) 7.2–7.3 was observed during high-frequency electrical stimulation in exercise-trained cardiomyocytes, whereas control cardiomyocytes became 0.1–0.2 pH units more acidic at the same stimulation frequencies, an effect that was attributed to increased sodium/hydrogen exchanger in exercise-trained cardiomyocytes [21], which shifts H^+ out of the cell and thus maintains normal intracellular pH. This favors Ca^{2+} sensitivity because acidity reduces Ca^{2+} activation of contraction, possibly due to H^+ competing with Ca^{2+} for troponin binding [35,36]. Since reduced pH in control cardiomyocytes was not observed during low-stimulation frequencies (low heart rates), it cannot explain the exercise training-induced increase in contractility at those low-stimulation frequencies, but it may be an important adaptation at high-stimulation frequencies and thus high heart rates, such as during intense exercise.

Actin–myosin cross-bridge

An exercise training-induced increase in atrial myosin light chain-1 has been linked to the myofilament Ca^{2+} sensitization [37], found after screening for a wide array of potential target genes, and then confirmed by targeted and selective protein expression studies. Although this study did not prove causation, others have strongly linked atrial myosin light chain-1 to myofilament Ca^{2+} sensitivity [38,39]. Other post-translational or epigenetic modifications to myofilament regulatory subunits or peptide isoforms that regulate actin–myosin cross-bridge formation and binding have also been suggested as plausible mechanisms [40–43].

Contraction–relaxation

Given the close link between cardiomyocyte intracellular Ca^{2+} handling and myofilament Ca^{2+} sensitivity and the resulting contractile function, the described Ca^{2+} effects following exercise training immediately suggest that contractile function also should be enhanced, and this is indeed the case. The same exercise training that leads to increased bioavailability of and responsiveness to Ca^{2+} also leads to improvements in cardiomyocyte contraction–relaxation and cardiac pump capacity, quantified by increased magnitude of contraction or fractional shortening in the cardiomyocyte [12,18–24,44] and increased stroke volume or cardiac output in the whole heart [1,2,45,46], as well faster rates of contractions and relaxations [12,18–24,47]. In particular, the exercise training-induced improvement to cellular contractile function, ability, and capacity has been almost uniformly and unanimously increased, with little discrepancy between different studies [45,46]. However, a caveat that

should be acknowledged is that these studies have been performed in unloaded cardiomyocytes that are not attached to other cells, which does not fully represent the in vivo situation in which pull from attached neighboring cells would oppose the intrinsic shortening during a contraction and facilitate relengthening during relaxation, but this is often the available medium with which to study contractile behavior in the single cell. With that in mind, these studies have provided good evidence that regular exercise training 1) increases cardiomyocyte contraction by up to ~50%, typically quantified by measuring fractional shortening [12,18–24,44–46]. 2) Increases cardiomyocyte rate of contraction–relaxation by up to ~30%; specifically, increased rate of relaxation remains a consistent finding, whereas rate of contraction has increased in some, but not all studies [12,18–24,45–47]. 3) Increased force and power development by up to ~60%, investigated under conditions where the cardiomyocyte is loaded [32,45,46].

Ca^{2+}-induced myocardial hypertrophy

The physiologic stress over a prolonged period of time that exercise represents not only enhanced intracellular Ca^{2+} handling and contractility of the cardiomyocyte, independent of cell size, but also enlarges the cardiomyocyte length and width [2,8,18–21,48,49] in a manner that leads to myocardial and cardiac hypertrophy [1,2,50], especially in the left ventricle. This also enhances contractile capacity through parallel deposition of sarcomeres. The molecular basis for physiologic hypertrophy following exercise training involves activation of both gene transcription [1,2,8,51–53] and protein translation [49,54,55] as well as post-translational modifications [56,57], for which other reviews provide comprehensive insight [1,2,8], but a particular mode of activation that involves intracellular Ca^{2+} should be mentioned here, that being excitation–transcription coupling [58]. In this scheme, Ca^{2+} either residing near or around the nucleus or 'spills over' from ECC to enter the nuclear vicinity, initiates signal pathways that enhance transcription of muscle hypertrophy genes:

Ca^{2+}–calmodulin-dependent protein kinase

CaMK activated by intracellular Ca^{2+}-bound calmodulin phosphorylates class-II nuclear histone deacetylase, which has been linked to postexercise training DNA demethylation and thereby enhanced nuclear myocyte enhancer factor transcription with downstream induction of muscle-specific gene programs and thus hypertrophy [8,9,58–60]. In this scenario, CaMK may induce gene transcription signaling that ultimately leads to physiologic muscle growth, even though the dominant evidence suggests that CaMK activation induces a fetal gene program that leads to pathologic, rather than physiologic, remodeling [9,58]. It should though be noted that the downstream effects of CaMK signaling may not only depend on expression and activity levels, but also post-translational modification of CaMK or its splice variants or localization within the cell. Differential effects due to such variations have already been shown in several models of cardiac remodeling and hypertrophy [61], but have not yet been studied in the context of exercise training.

Calcineurin

Also activated by Ca^{2+}, calcineurin dephosphorylates the transcription factor nuclear factor of activated T cells, which translocates to the nucleus and activates muscle-specific gene transcription [62]. However, the role of calcineurin in modulating exercise training-induced hypertrophy in the heart is also a matter of controversy [63], since the two available studies of its specific role in cardiac physiological hypertrophy, within only a few years of each other, reported diametrically opposite findings: one demonstrating that calcineurin was involved in exercise training-induced left ventricular growth [64] and the other demonstrating that it was not involved in exercise training-induced left ventricular growth [65], and in fact, if anything, it reduced its activity during hypertrophy development during the course of the exercise training. However, this aspect is further complicated by a subcellular CaMK–calcineurin interplay, as it has also been shown that in situations where normal CaMK activation is prevented, calcineurin may instead initiate to generate the same or comparable cellular effect because the CaMK inactivation also prevents CaMK-specific phosphorylation of the calcineurin autoinhibitory residue, and one situation where this occurs is after swim-exercise training [66], and although no physiologic benefit was observed as a result of this CaMK–calcineurin interaction, neither were maladaptive effects observed.

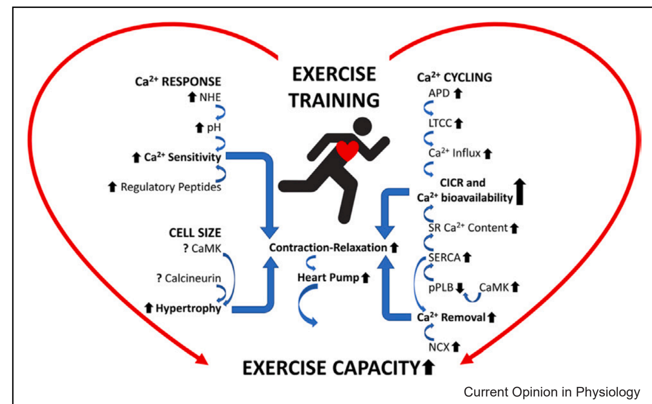
Thus, not all aspects of Ca^{2+}-linked hypertrophy signaling have been elucidated or fully established yet, though its presence seems clear [58]. Given Ca^{2+} is readily available in the cardiomyocyte due to its cycling during exercise, it might evolutionary have been favored for adaptation to facilitate the very contractions that release and cycle it, but when investigated, the available evidence is not clear on either mechanism and role, and it has also been linked to pathologic remodeling. It remains, for instance, possible that different Ca^{2+} loads or time courses of stimulation, acute and brief with exercise training versus chronic and sustained with pathology, or effects specific to intracellular loci, yield different transcriptional, translational, and post-translational outcomes. Incidentally, the pre-eminent molecular pathway for modulating physiological cardiac hypertrophy (insulin-like growth factor–phosphatidylinositol-3-kinase–Akt) that also has the greatest amount of evidential weight behind it [2,8,54,55], does not appear to be regulated by Ca^{2+}, but it may have regulation *to* Ca^{2+}

cycling, that is, several studies have shown that Akt-dependent phosphorylation stabilizes and enhances LTCC [67] and SERCA [68] functions, which thereby increases contractile function.

Detrimental effects of Ca^{2+}

May there be any detrimental effects ensuing from increased Ca^{2+} cycling and handling in the cardiomyocyte or the heart as a whole? Unfortunately, yes! The issue at hand is that Ca^{2+} channels and Ca^{2+}-sensitive regulatory proteins involved in cardiac ECC and contraction may also provide an aberrant Ca^{2+} substrate that may increase the probability of life-threatening arrhythmias. Mutations in the RyR macromolecular complex [69,70] or LTCC [71] often precede such arrhythmias. Commonly, they occur without a known, identifiable source or previous diagnosis. Recent studies have suggested that alterations to SR Ca^{2+} loading and the balance between SR Ca^{2+} uptake (SERCA) and leak (RyR), such as may be precipitated by strenuous physical effort and exercise training, may increase the Ca^{2+}-linked arrhythmogenic potential [72], albeit it may be difficult to discriminate between exercise-induced arrhythmias that occur due to higher rate of ECC and Ca^{2+} cycling versus those that occur in response to sympathetic or catecholaminergic stimulation, as they also associate with exercise [73]. Nonetheless, a hypothesis has been suggested that prolonged, chronic exercise training, perhaps at high physical and/or emotional stress, may cause arrhythmic episodes and sudden death, as those well-covered by mass media in football (soccer) and endurance events such as roadrace cycling and marathons. This phenomenon has been observed in heart disease patients [74], but also in healthy young athletes [75]. As Ca^{2+} dysregulation may also occur in seemingly healthy hearts, so may those arrhythmias [69–71]. A recent report [14] has suggested a mechanism for this, in which 3 months of swim-exercise training in rats, a program that was effective in evoking all expected phenotype adaptations, resulted in a biologically (and statistically) significant increase in SR Ca^{2+} content. In the face of lowered resting heart rate, also a common finding after prolonged endurance exercise training, this increased spontaneous Ca^{2+}-release events and extrasystoles [14]. Since such spontaneous Ca^{2+}-release events and extrasystoles have been implicated in generating ventricular arrhythmias [16,69–72], and although this study did not assess arrhythmias per se, it follows that the described exercise training-induced adaptation generates an arrhythmogenic trigger source in an otherwise healthy heart. Thus, normal exercise training-associated remodeling of cardiomyocyte Ca^{2+} handling may therefore also explain the occurrences of life-threatening arrhythmias that have occasionally been observed in endurance athletes [75]. To provide a balanced perspective though, it is important to note that

Figure 1



Summary of exercise training-induced adaptations to cardiomyocyte calcium (Ca^{2+}) handling. APD: action potential duration; NCS: sodium/Ca^{2+} exchanger; NHE: sodium/hydrogen exchanger; pPLB: phosphorylated phospholamban.

Ca^{2+}-linked or other arrhythmias generated while exercising or in endurance exercise-trained persons without known underlying arrhythmogenic causes are extremely rare, and the generally held belief is that the physiologic, clinical, and well-being benefits of exercise training outweigh the potential risk, in healthy [75–77] as well as in disease [78].

In contrast to the above, several studies from different laboratories have also shown that exercise training confers a preventive, antiarrhythmogenic adaptation, as it reduces or prevents arrhythmogenic spontaneous SR Ca^{2+}-release events, measured as RyR-mediated Ca^{2+} sparks [79] and waves [80] as well as actual Ca^{2+}-linked arrhythmias [81]. This was already at least partly assumed when exercise training was shown to decrease diastolic $[Ca^{2+}]$ [12,18–21,23,24], which would be expected to decrease the risk of arrhythmogenic Ca^{2+}-release events, but not shown until more recently. The proposed mechanisms for this include increased SERCA [23–25], which would preventively stabilize $[Ca^{2+}]$ in diastole and thereby reduce stimulation for spontaneous RyR Ca^{2+}-release events, and upheld cytoskeletal and transverse tubule integrity [82], which also reduces arrhythmogenicity.

Conclusive remarks and conclusion

Not all aspects of the Ca^{2+}-linked adaptations are as of yet fully understood. For instance, although Ca^{2+} exists in all compartments of the cell [3], its modulation may not be ubiquitous or uniform across the cell. Different Ca^{2+} gradients may be created depending on intracellular architecture, or organization of Ca^{2+}-associated proteins, such that different cell loci may experience different and localized Ca^{2+} signaling events.

A case in point would be the site-specificity and differential effects of CaMK signaling, whereby exercise training increases PLB phosphorylation to increase SERCA Ca^{2+} uptake function in the SR [23,25], whereas CaMK inhibition reduces Ca^{2+} handling and contractility [23,83], yet transgenic overexpression of CaMK leads to Ca^{2+} dysfunction [9,12], and albeit this is reversed by exercise training [12], it points also to a pathologic role of CaMK. In yet another scenario, exercise training reduces CaMK-dependent RyR phosphorylation to the degree that it reduces arrhythmias in mice with a RyR-caused catecholaminergic polymorphic ventricular tachycardia missense mutation [81], whereas in other studies, an exercise training-induced reduction in spontaneous arrhythmogenic Ca^{2+}-release events [79,80] could not be linked to changes in RyR or other Ca^{2+} cycling proteins [79]. Thus, we seem to understand the main points of Ca^{2+}-linked exercise training adaptations in the heart, but some of the finer details are not quite as clear. As such, further studies are called upon, especially with a greater spatiotemporal resolution for localized events and patterns that shape whole-cell and whole-heart responses to exercise training. For this, continued development of, for example, confocal or multiphoton microscopy techniques in the isolated cell or in vivo heart, might provide clarity on localized Ca^{2+} effects and modulators such as CaMK and calcineurin, including crosstalk, the role of mitochondria in beat-to-beat Ca^{2+} handling, and the involvement of indirect adjacent but potentially important regulatory factors linked to membrane potential, autophagy and maintenance of cell integrity, and energy substrate availability [1,2,8,84–86].

It is however clear that Ca^{2+} plays a major role in the postexercise training adaptation, as highlighted in this review and diagrammatically summarized in Fig. 1. Ca^{2+}-induced and Ca^{2+}-modulated adaptation, whether this is ECC and beat-to-beat Ca^{2+} handling with reshaping or increasing the intracellular Ca^{2+} release or myofilament responsiveness to Ca^{2+}, or excitation–transcription coupling with either initiation or silencing of gene transcription, enhances contractile function and capacity, and therefore heart pump function and whole-body exercise and physical capability, in both health and disease. When analyzed by correlation or regression models, there is a close relationship and a high level of interdependency between cellular cardiac adaptations that include all of intracellular Ca^{2+} handling, contractility, and size parameters on the one side, and whole-heart or whole-body parameters on the other side, following exercise training [18,19,21,48]. Furthermore, the adaptation is dynamic, with magnitude of change being determined by the magnitude of the stress (exercise) stimulus, such as intensity, frequency, duration, and even cessation of the exercise being carried out. This has been indicated by comparing different studies utilizing different exercise training protocols, but more importantly also by studies directly comparing different exercise training regimens to one another. Finally, different modes of exercise, such as resistance exercise training, may also confer specific but disparate cardiac benefits, and that may or may not be Ca^{2+}-dependent [87], but which may provide parallel benefits that altogether contribute to a better functioning heart.

CRediT authorship contribution statement

Ole J Kemi: Conceptualization, Writing – review & editing.

Conflict of interest statement

Nothing declared.

Data Availability

No data were used for the research described in the article.

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