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Aerobic interval training enhances cardiomyocyte contractility and Ca²⁺ cycling by phosphorylation of CaMKII and Thr-17 of phospholamban

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Abstract

Cardiac adaptation to aerobic exercise training includes improved cardiomyocyte contractility and calcium handling. Our objective was to determine whether cytosolic calcium/calmodulin-dependent kinase II and its downstream targets are modulated by exercise training. A six-week aerobic interval training program by treadmill running increased maximal oxygen uptake by 35% in adult mice, whereupon left ventricular cardiomyocyte function was studied and myocardial tissue samples were used for biochemical analysis. Cardiomyocytes from trained mice had enhanced contractility and faster relaxation rates, which coincided with larger amplitude and faster decay of the calcium transient, but not increased peak systolic calcium levels. These changes were associated with reduced phospholamban expression relative to sarcoplasmic reticulum calcium ATPase, and constitutively increased phosphorylation of phospholamban at the threonine 17, but not at the serine 16 site. Calcium-calmodulin-dependent kinase IIδ phosphorylation was increased at Threonine 287, indicating activation. To investigate the physiological role of calcium/calmodulin-dependent kinase IIδ phosphorylation, this kinase was blocked specifically by autocamtide-2 related inhibitory peptide II. This maneuver completely abolished training-induced improvements of cardiomyocyte contractility and calcium handling, and blunted, but did not completely abolish the training-induced increase in Ca²⁺ sensitivity. Also, inhibition of calcium/calmodulin-dependent kinase II reduced the greater frequency-dependent acceleration of relaxation that was observed after aerobic interval training. These observations indicate that calcium/calmodulin-dependent kinase IIδ contributes significantly to the functional adaptation of the cardiomyocyte to regular exercise training.

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Keywords

Calcium; Contractility; Myocytes; CaMK; SERCA; PLB

1. Introduction

We have previously shown that aerobic capacity is closely related to cardiomyocyte contractility and calcium handling in training [1,2], detraining [3], post-infarction heart failure [4] and genetic selection of high versus low exercise capacity [5]. It has also been demonstrated that exercise-induced improvement of contractile function in cardiomyocytes may be linked to increased activity of the cardiac sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA-2a) both in healthy rats [2,6] and in rats with post-infarction heart failure [4]. Uptake of Ca^{2+} into sarcoplasmic reticulum (SR) by SERCA-2a is an important determinant of the rate of cardiac muscle relaxation. It also determines the Ca^{2+} loading of the SR and thus the amount of Ca^{2+} available for release during cardiomyocyte contraction [7]. SERCA-2a is regulated by phospholamban (PLB); unphosphorylated PLB binds to SERCA-2a and inhibits its activity, whereas phosphorylation removes PLB from SERCA-2a and increases Ca^{2+} uptake rate. Mainly, PLB is phosphorylated by cAMP-dependent protein kinase A (PKA) at serine (Ser)-16 and by Ca^{2+} /calmodulin dependent kinase II (CaMKII) at threonine (Thr)-17 [8]. There are also indications of a PLB-independent action of CaMKII upon SERCA-2a that appears to increase diastolic SR Ca^{2+} re-uptake [9].

It is widely accepted that Ser-16 phosphorylation is important for β -adrenergic modulation of cardiac contractility and relaxation, and that CaMKII-mediated Thr-17 PLB phosphorylation increases at high heart rates [10]. Furthermore, CaMKII has been functionally implicated in the mediation of frequency-dependent acceleration of relaxation (FDAR) that contributes to optimal diastolic ventricular filling at increased heart rates [9], even in PLB-knockout mice [11].

The purpose of this study was to investigate whether CaMK-mediated changes in Ca^{2+} signaling contribute to the exercise-mediated effects on inotropy and lusitropy in mice cardiomyocytes.

2. Material and methods

2.1 Experimental model and aerobic interval training

In total, 62 female C57BL/6J mice (Møllegaards Breeding Center, Lille Skensved, Denmark), 19-21 g and 8 weeks old at inclusion were randomized to sedentary control or regular aerobic interval training on an inclined (25°) treadmill for 6 weeks, 5 days/week, 1.5 hours/day. After a 20 minute warm-up at 50-60% of maximal oxygen uptake ($\text{VO}_{2\text{max}}$), the mice alternated between 8 and 2 min intervals at 85-90% and 50-60% of $\text{VO}_{2\text{max}}$, respectively. Thus, since the treadmills were quickly accelerated or decelerated to the preferred speed, accumulated high-intensity running was performed for 56 minutes. $\text{VO}_{2\text{max}}$ was assessed during exhaustive treadmill running in a custom-made metabolic chamber, before and after the training period, and at the start of every training week, to adjust relative intensity in the training group. Animals were sacrificed 24 hours after the last exercise bout and 1 week after the last $\text{VO}_{2\text{max}}$ test, to avoid effects of acute exercise. Procedures for handling animals, as well as training and testing $\text{VO}_{2\text{max}}$ were previously published [12]. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Experimental protocols were approved by the Norwegian Animal Research Ethics Council.

2.2 Cardiomyocyte contractility and Ca^{2+} handling

Single cardiomyocytes from the left ventricle were isolated with a Krebs-Henseleit Ca^{2+} -free buffer, collagenase type 2, and CaCl_2 added stepwise to 1.2 mmol/L, as previously described [12,13]. Fura-2/AM-loaded (2 $\mu\text{mol/L}$, Molecular Probes, Eugene, OR) myocytes were stimulated with bipolar electrical pulses at 0.5, 2, and 5 Hz on an inverted epifluorescence microscope (Diaphot-TMD, Nikon, Tokyo, Japan), whereupon cell shortening was recorded by video edge-detection (Model 104, Crescent Electronics, Sandy, UT) and intracellular $[\text{Ca}^{2+}]_i$ ($[\text{Ca}^{2+}]_i$) was probed by counting 510 nm emission after exciting with alternating 340 and 380 nm wavelengths (Photon Technology International, Lawrenceville, NJ). The ratio signal of the two excitation wavelengths was subsequently converted to $[\text{Ca}^{2+}]_i$ by measuring $R_{\min}$ and $R_{\max}$ in permeabilized cells and assuming a K_d of 200 nM [2]. During the stimulation protocol, cells were continuously perfused with HEPES buffer (1.2 $\mu\text{mol/L}$ Ca^{2+} , 37° C). In separate experiments, cells were incubated 30 min before and during stimulation protocol with autamtide-2 related inhibitory peptide II (AIP; 10 $\mu\text{mol/L}$; Calbiochem, San Diego, CA) to target CaMK. From each condition, 10 stable, consecutive contractions at each stimulation frequency were averaged.

2.3 Cytosolic extracts and SR vesicle preparation

Frozen left ventricles from trained and sedentary mice were homogenized in lysis buffer (30 mmol/L Tris-HCl, pH 7.5, 150 mmol/L KCl, 300 mmol/L Sucrose and 10 mmol/L NaF) and centrifuged for 20 min at 8000 g at 4° C; the supernatant was further ultracentrifuged 30 min at 100,000 g at 4° C. Cytosolic extract was defined as supernatant while the pellet was considered as SR/membrane-enriched fraction [14].

2.4 SDS-PAGE and immunoblotting

30 μg of cytosolic and SR extracts from both trained and sedentary mice were loaded on 10% SDS-PAGE for detection of CaMKII, 15% SDS-PAGE was used for PLB detection. Proteins were blotted on nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) and membranes were blocked with TBS-T/milk (50 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 0.1% v/v Tween-20, 5% w/v non-fat dried milk) for 1 hour at room temperature, followed by 1 hour room temperature incubation with the following antibodies: total PLB and phospho-Thr-17-PLB antibodies (Badrilla, Leeds, UK), phospho-Ser-16-PLB antibody (Upstate, Charlottesville, VA), phospho-Thr-287-CaMKII (Affinity Bioreagents, Golden, CO) and total CaMKII, kindly provided by Prof. Harold A. Singer (Albany Medical College, NY). As secondary antibodies, horseradish peroxidase-conjugate goat anti-rabbit and anti-mouse were used (Amersham, Buckinghamshire, UK). Membranes were developed using the enhanced chemiluminescence (ECL) detection system (Amersham).

2.5 Statistics

Data are presented as mean $\pm$ SD, with significance level $p < 0.05$. Comparisons were made using ANOVA with Scheffe post-hoc tests.

3. Results

3.1 Aerobic capacity, cardiomyocyte contractility, and Ca^{2+} handling

Aerobic interval training induced a marked adaptive response in aerobic capacity and cardiomyocyte contractile function and Ca^{2+} handling. $\text{VO}_{2\max}$ increased by 35% (Figure 1A), and isolated cardiomyocytes were ~20% longer and ~30% wider in trained mice than in sedentary controls (117 ± 12 vs. 98 ± 10 and 27 ± 6 vs. 21 ± 4 μm cell length and width for trained and sedentary mice, respectively; $p < 0.01$ for both comparisons). Typical examples of cardiomyocyte shortening and the Ca^{2+} transient are shown in Figure 1B and C,

respectively. Fractional shortening and Ca^{2+} transient amplitude were not significantly different between trained and sedentary groups at 0.5 Hz stimulation, but at physiological contraction rates (2-5 Hz), fractional shortening and Ca^{2+} transient amplitudes were larger in trained mice; at 5 Hz stimulation, fractional shortening and Ca^{2+} transient amplitude were 60% and 50% larger, respectively, after aerobic interval training (Figure 1D-G). The larger Ca^{2+} transient amplitude occurred despite reduced systolic and diastolic Ca^{2+} levels; the reduction in diastolic Ca^{2+} level was greater than in systolic Ca^{2+} level (example traces on Figure 1C, and Figure 1F). However, diastolic shortening during the stimulation protocol was not different between cardiomyocytes from sedentary and trained mice (Figure 1G). Since the amplitude of the fractional shortening increased with training, but peak systolic $[\text{Ca}^{2+}]_i$ did not, it indicates that myofilament sensitivity to Ca^{2+} has shifted with aerobic interval training. Therefore, we plotted fractional shortening during systole (corrected for diastolic shortening during increased stimulation frequencies) to peak systolic Ca^{2+} in each cardiomyocyte, and found that this index of Ca^{2+} sensitivity increased by ~20% after training (Figure 3C). Moreover, increased Ca^{2+} sensitivity was also indicated during diastole (Figure 3D).

3.2 Ca^{2+} -regulating proteins related to SR function

SERCA-2a protein levels in SR extracts increased by ~25% after aerobic interval training (Figure 2A), whereas PLB remained unchanged (Figure 2B). This alone reduces PLB-to-SERCA-2a protein ratio, such that SERCA-2a is likely to be less inhibited in hearts from exercise trained mice. In hearts from trained mice, phosphorylation was selectively elevated at Thr-17 (Figure 2C), whereas phosphorylation at Ser-16 remained unchanged (Figure 2D). Hearts from trained mice also had elevated phosphorylation at Thr-287 of CaMKII δ (the main cardiac CaMK isoform), while total protein amount was unchanged (Figure 2E). Since CaMKII is known to phosphorylate Thr-17 PLB [8], these observations suggest that adaptation to training includes elevated SERCA-2a activity and improved inotropy and lusitropy because of CaMKII activation in cardiomyocytes from trained mice.

3.3 Improved cardiomyocyte fractional shortening and Ca^{2+} handling is mediated by CaMKII

To further investigate the role of CaMKII in mediating the exercise training-induced adaptive improvement in contractility and Ca^{2+} handling, we also studied the response of cardiomyocytes to CaMKII blockade. In isolated cardiomyocytes, the training-induced increase in fractional shortening was completely blunted after pre-incubation with AIP (CaMKII inhibitor), whereas no change occurred in cells from sedentary mice (Figure 3A). Parallel changes occurred in the Ca^{2+} transient; in trained cardiomyocytes, acute treatment with the same kinase inhibitor reduced amplitude to a level similar to controls (Figure 3B). When plotting systolic shortening to peak systolic Ca^{2+} (Figure 3C) and maximal diastolic shortening to diastolic Ca^{2+} (Figure 3D), it was revealed that inhibition of CaMKII blunted, but did not completely remove the training-induced improvement on Ca^{2+} sensitivity during both systole and diastole, respectively. No effects were observed in cardiomyocytes from sedentary mice.

3.4 Cardiomyocyte diastolic function and frequency-dependent acceleration of relaxation (FDAR)

As expected, time to 50% cell re-lengthening decreased with increasing stimulation frequencies in both trained and control cardiomyocytes. No difference between groups was observed at 0.5 Hz whereas time to re-lengthening was shorter in trained cardiomyocytes at 2 and 5 Hz ($p < 0.01$). The difference in re-lengthening between sedentary and exercised groups was larger at 5 Hz than at 2 Hz ($p < 0.03$). Interestingly, the frequency-dependent acceleration of relaxation (FDAR) with increasing stimulation frequencies, as well as the

differences between trained and untrained hearts, were absent after pre-incubating cells with AIP (Figure 3E).

Changes in time to 50% Ca^{2+} decay paralleled those in time to 50% re-lengthening. Time to 50% decay of the Ca^{2+} transient decreased with increasing stimulation frequencies in both trained and control cardiomyocytes. No difference between groups was observed at 0.5 Hz, but time to Ca^{2+} decay was shorter in trained cardiomyocytes at 2 and 5 Hz ($p < 0.01$, Figure 3F). Additionally, the difference in Ca^{2+} decay between trained and untrained hearts was larger at 5 than at 2 Hz ($p < 0.01$). The changes in Ca^{2+} handling disappeared after CaMK inhibition (Figure 3D & E). These findings indicate that a major component of the training-induced increase in cardiomyocyte contractility is related to changes in Ca^{2+} handling, specifically in CaMKII-mediated regulation of SERCA-2a activity.

4. Discussion

This is the first study to characterize the effects of regular aerobic interval training on Ca^{2+} signaling in mouse hearts. The main finding was that training reduces the PLB-to-SERCA-2a protein expression ratio and constitutively increases phosphorylation of cytosolic Thr-287 CaMKII δ and its downstream target Thr-17 PLB in cardiomyocytes. The physiological importance of CaMK for exercise training-induced improvements in inotropy and lusitropy was confirmed in electrically stimulated cardiomyocytes.

4.1 Aerobic interval training up-regulates SERCA-2a, PLB and CaMK

Cardiac output is a major determinant of oxygen transport and aerobic metabolism; hence, increasing cardiac output is an essential adaptation to aerobic endurance training [15]. The cellular basis for this involves improved contractile function of the cardiomyocyte, which we have demonstrated occurs with exercise training in rats [1-3]. The present study confirms that these changes occur in cardiomyocytes isolated from exercise trained mice, and identify sub-cellular mechanisms that contribute to improved inotropy and lusitropy.

Aerobic interval training increased protein expression of SERCA-2a without changing total PLB protein levels, and thereby reduced the PLB/SERCA-2a ratio. Since PLB is the primary inhibitor of SERCA-2a [8,16], the reduced ratio will increase the Ca^{2+} sensitivity and the enzyme activity (V_{max}) of SERCA-2a. Increased SERCA-2a mRNA [17] and protein [2] expressions have previously been reported in exercise trained rats. Further increases in the Ca^{2+} sensitivity (reduced affinity for Ca^{2+} , K_m) of SERCA-2a would result from the increased phosphorylation of the Thr-17 of PLB [16,18], although it has also been suggested that Thr-17 phosphorylation of PLB also may increase the V_{max} of SERCA-2a [19,20].

The cause of the increase in phosphorylation status is not clear, but the current study observed an increase in the phosphorylation of the Thr-287 residue of cytosolic CaMKII δ , while total CaMKII δ protein expression was unchanged. Since CaMKII is known to specifically phosphorylate Thr-17 PLB, these data suggest a causal link. This hypothesis was supported by the effect of the specific CaMK-inhibitor AIP, which was able to substantially blunt the exercise training-induced increase in fractional shortening and Ca^{2+} transient amplitude. Previous work has shown that CaMKII has a series of targets in the cell, including the L-type Ca^{2+} channel, the ryanodine receptor 2 (RyR2), and SERCA-2a [8,9]. Hence, inhibiting CaMK with AIP may have limited the inotropic response of exercise by acting on a series of sites in the excitation-contraction coupling process that collectively abolished the training-induced effects. In contrast to Thr-17 PLB, the Ser-16 residue (PKA site) does not appear to be modulated by aerobic interval training.

4.2 Myofilament Ca^{2+} sensitivity

Improved myofilament sensitivity to Ca^{2+} is an important adaptation of the rat heart to regular exercise training [1-3,21], and is now also indicated in the mouse heart. The CaMK-blockade maneuver in trained cardiomyocytes caused Ca^{2+} sensitivity to revert toward levels closer to sedentary controls; hence, suggesting that CaMK partly modulates the training-induced improvement in Ca^{2+} sensitivity. A proposed mechanism for improved Ca^{2+} sensitivity has been that troponins T [22] and I [23] undergo a shift in the isoforms expressed and that atrial myosin light chain 1 increases expression levels [24]. It has also been shown that CaMKII can act to mediate troponin I function [8] and the promoter-region of the atrial myosin light chain-1 gene [25]. It now appears that CaMKII may be able to acutely induce a shift in the myofilament sensitivity to Ca^{2+} to a more energetically favorable state after exercise training, although other studies have suggested that PKA mediates Ca^{2+} sensitivity [26]. This is conceivable also in the current study, since CaMKII-blockade only partially blunted the training-induced increase in Ca^{2+} sensitivity.

4.3 Aerobic interval training improves cardiomyocyte lusitropy

Aerobic interval training-induced improvement of diastolic function was demonstrated by the faster rates of cardiomyocyte re-lengthening and decay of the Ca^{2+} transient, in line with exercise trained rats [1-3]. These effects were partly dependent upon CaMKII being available in the cytosol, as CaMK-inhibition by AIP partly removed the exercise training-induced effects when cells were paced at frequencies as close to physiological mouse heart rates as experimentally possible. Given the proposed effect on Ca^{2+} re-sequestration (see above), CaMKII δ and its downstream targets appear to enhance cardiomyocyte SR Ca^{2+} uptake after exercise training.

4.4 Frequency-dependent acceleration of relaxation (FDAR)

FDAR ensures appropriate ventricular filling at high heart rates, and results from accelerated SR Ca^{2+} uptake independent of Ca^{2+} removal out of the cell [27]. However, the contributions of PLB and CaMKII to FDAR remain controversial [28]. No differences were evident between sedentary and exercise trained for re-lengthening and Ca^{2+} decay rates at low stimulation frequencies, but a clear training-induced acceleration of relaxation was clear at high stimulation frequencies. The fact that CaMK-inhibition abolished this training-induced effect, suggests that CaMK-dependent Thr-17 PLB phosphorylation regulates FDAR and that this is mediated in response to regular exercise training. Our hypothesis is strengthened by reports suggesting that CaMKII-mediated phosphorylation during low stimulation frequencies is small, but increases substantially when stimulation frequencies increase [9], and that Thr-17, but not Ser-16, is the phosphorylation residue on PLB that mediates FDAR [10], in line with our results. Since Thr-287 CaMKII and Thr-17 PLB constitutively increased phosphorylation status, as observed by the immunoblots 24 hours after the last exercise bout, it suggests that physiological heart rates or equivalent stimulation frequencies are necessary to elicit the CaMKII-mediated effect. However, FDAR is robustly present in PLB-knockout mice, where it remains sensitive to CaMKII inhibitors [11,29], suggesting that other regulatory targets may be mediated by CaMK; perhaps compensatory to loss of PLB function, whereas other studies did not detect any effects of CaMK inhibitors on FDAR in rat cardiomyocytes [28]. Hence, the mechanistic understanding of FDAR is incomplete.

4.5 Conclusion

We have extended previous studies of exercise training-induced changes of cardiomyocyte contractility and Ca^{2+} handling in rats to mice. Improved inotropy and lusitropy appear as general adaptations to exercise training in cardiomyocytes across species. These changes in

cardiomyocyte contractility in response to exercise training appear to be partly caused by improved SR Ca^{2+} uptake via a combination of reduced PLB/SERCA-2a protein ratios and by phosphorylation of Thr-17 PLB. Increased myofilament sensitivity also contributes to the final positive inotropic response. Since the Thr-287 residue of cytosolic CaMKII δ increased phosphorylation upstream of Thr-17 PLB, it suggests a regulatory pathway modulated by exercise training. Acute inhibition of CaMK abolished the training-induced increase in fractional shortening by reversing the effects on intracellular Ca^{2+} handling and myofilament Ca^{2+} sensitivity.

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References

1. Kemi OJ, Haram PM, Loennechen JP, Osnes JB, Skomedal T, Wisloff U, et al. Moderate vs. High intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function. *Cardiovasc Res* 2005;67:161–72. [PubMed: 15949480]
2. Wisloff U, Loennechen JP, Falck G, Beisvag V, Currie S, Smith GL, et al. Increased contractility and calcium sensitivity in cardiac myocytes isolated from endurance trained rats. *Cardiovasc Res* 2001;50:495–508. [PubMed: 11376625]
3. Kemi OJ, Haram PM, Wisloff U, Ellingsen O. Aerobic fitness is associated with cardiomyocyte contractile capacity and endothelial function in exercise training and detraining. *Circulation* 2004;109:2897–904. [PubMed: 15173028]
4. Wisloff U, Loennechen JP, Currie S, Smith GL, Ellingsen O. Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca^{2+} sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovasc Res* 2002;54:162–74. [PubMed: 12062372]
5. Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* 2005;307:418–20. [PubMed: 15662013]
6. Tate CA, Taffet GE, Hudson EK, Blaylock SL, McBride RP, Michael LH. Enhanced calcium uptake of cardiac sarcoplasmic reticulum in exercise-trained old rats. *Am J Physiol* 1990;258:H431–5. [PubMed: 2137986]
7. Periasamy M, Huke S. SERCA pump level is a critical determinant of Ca^{2+} homeostasis and cardiac contractility. *J Mol Cell Cardiol* 2001;33:1053–63. [PubMed: 11444913]
8. Maier LS, Bers DM. Calcium, calmodulin, and calcium-calmodulin kinase II: heartbeat to heartbeat and beyond. *J Mol Cell Cardiol* 2002;34:919–39. [PubMed: 12234763]
9. Picht E, DeSantiago J, Huke S, Kaetzel MA, dedman JR, Bers DM. CaMKII inhibition targeted to the sarcoplasmic reticulum inhibits frequency-dependent acceleration of relaxation and Ca^{2+} current facilitation. *J Mol Cell Cardiol* 2007;42:196–205. [PubMed: 17052727]
10. Hagemann D, Kuschel M, Kuramochi T, Zhu W, Cheng H, Xiao RP. Frequency-encoding Thr¹⁷ phospholamban phosphorylation is independent of Ser¹⁶ phosphorylation in cardiac myocytes. *J Biol Chem* 2000;275:22532–6. [PubMed: 10825152]
11. Li L, Chu G, Kranias EG, Bers DM. Cardiac myocyte calcium transport in phospholamban knockout mouse: relaxation and endogenous CaMKII effects. *Am J Physiol* 1998;274:H1335–47. [PubMed: 9575939]
12. Kemi OJ, Loennechen JP, Wisloff U, Ellingsen O. Intensity-controlled treadmill running in mice: cardiac and skeletal muscle hypertrophy. *J Appl Physiol* 2002;93:1301–9. [PubMed: 12235029]

13. Lim CC, Apstein CS, Colucci WS, Liao R. Impaired cell shortening and relengthening with increased pacing frequency are intrinsic to the senescent mouse cardiomyocyte. *J Mol Cell Cardiol* 2000;32:2075–82. [PubMed: 11040110]
14. Ji Y, Li B, Reed TD, Lorenz JN, Kaetzel MA, Dedman JR. Targeted inhibition of Ca^{2+} /calmodulin-dependent protein kinase II in cardiac longitudinal sarcoplasmic reticulum results in decreased phospholamban phosphorylation at threonine 17. *J Biol Chem* 2003;278:25063–71. [PubMed: 12692124]
15. Richardson RS, Harms CA, Grassi B, Hepple RT. Skeletal muscle: master or slave of the cardiovascular system? *Med Sci Sports Exerc* 2000;32:89–93. [PubMed: 10647534]
16. MacLennan DH, Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* 2003;4:566–77. [PubMed: 12838339]
17. Buttrick PM, Kaplan M, Leinwand LA, Scheuer J. Alterations in gene expression in the rat heart after chronic pathological and physiological loads. *J Mol Cell Cardiol* 1994;26:61–7. [PubMed: 8196070]
18. Cantilina T, Sagara Y, Inesi G, Jones LR. Comparative studies of cardiac and skeletal sarcoplasmic reticulum ATPases. Effect of a phospholamban antibody on enzyme activation by Ca^{2+} . *J Biol Chem* 1993;268:17018–25. [PubMed: 8349590]
19. Antipenko AY, Spielman AI, Kirchberger MA. Comparison of the effects of phospholamban and jasmonate on the calcium pump of cardiac sarcoplasmic reticulum. Evidence for modulation by phospholamban of both Ca^{2+} affinity and $V_{\max}(\text{Ca})$ of calcium transport. *J Biol Chem* 1997;272:2852–60. [PubMed: 9006928]
20. Mattiazzi A, Hove-Madsen L, Bers DM. protein kinase inhibitors reduce SR Ca transport in permeabilized cardiac myocytes. *Am J Physiol* 1994;267:H812–20. [PubMed: 8067437]
21. Diffie GM, Seversen EA, Titus MM. Exercise training increases the Ca^{2+} sensitivity of tension in rat cardiac myocytes. *J Appl Physiol* 2001;91:309–15. [PubMed: 11408445]
22. Anderson PA, Greig A, Mark TM, Malouf NN, Oakeley AE, Ungerleider RM, et al. Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult, and failing heart. *Circ Res* 1995;76:681–6. [PubMed: 7534662]
23. Layland J, Cave AC, Warren C, Grieve DJ, Sparks E, Kentish JC, et al. Protection against endotoxemia-induced contractile dysfunction in mice with cardiac-specific expression of slow skeletal troponin I. *FASEB J* 2005;19:1137–9. [PubMed: 15855227]
24. Diffie GM, Seversen EA, Stein TD, Johnson JA. Microarray expression analysis of effects of exercise training: increase in atrial MLC-1 in rat ventricles. *Am J Physiol Heart Circ Physiol* 2003;284:H830–7. [PubMed: 12424097]
25. Woischwill C, Karczewski P, Bartsch H, Luther HP, Kott M, Haase H, et al. Regulation of the human atrial myosin light chain 1 promoter by Ca^{2+} -calmodulin-dependent signaling pathways. *FASEB J* 2005;19:503–11. [PubMed: 15791000]
26. van der Velden J, Merkus D, Klarenbeek BR, James AT, Boontje NM, Dekkers DH, et al. Alterations in myofilament function contribute to left ventricular dysfunction in pigs early after myocardial infarction. *Circ Res* 2004;95:e85–95. [PubMed: 15528471]
27. Bassani RA, Mattiazzi A, Bers DM. CaMKII is responsible for activity-dependent acceleration of relaxation in rat ventricular myocytes. *Am J Physiol* 1995;268:H703–12. [PubMed: 7864197]
28. Valverde CA, Mundina-Weilenmann C, Said M, Ferrero P, Vittone L, Salas M, et al. Frequency-dependent acceleration of relaxation in mammalian heart: a property not relying on phospholamban and SERCA2a phosphorylation. *J Physiol* 2005;562:801–13. [PubMed: 15528241]
29. DeSantiago J, Maier LS, Bers DM. Frequency-dependent acceleration of relaxation in the heart depends on CaMKII, but not phospholamban. *J Mol Cell Cardiol* 2002;34:975–84. [PubMed: 12234767]

Figure 1.

Panel A; Time course of the increase in maximal oxygen uptake (VO_{2max}) during 6 weeks of aerobic interval training by treadmill running, 5 days/week at 90-95% of VO_{2max} (TR, n=31) versus sedentary controls (SED, n=31). Panel B; Example traces of Cardiomyocyte shortening in SED and TR mice during 5 Hz electrical field stimulation, and panel C; respective intracellular $[Ca^{2+}]_i$ transient traces. Panel D; Cardiomyocyte fractional shortening in SED and TR mice during 5 Hz electrical stimulation, and panel E; respective $[Ca^{2+}]_i$ transient amplitudes. Panel F; systolic and diastolic $[Ca^{2+}]_i$ in TR and SED mice at 0.5-5 Hz electrical field stimulation, and panel G; respective relative cell lengths during diastole and systole. n=12 TR + 12 SED, 5-8 cells/mouse; data are means $\pm$ SD. Significant difference between TR vs. SED; *: $p<0.01$. Difference between 2-6 weeks of training vs. pretest; #: $p<0.01$.

Figure 2.

Panel A; protein levels of sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA-2a). Panel B; protein levels of total phospholamban (PLB). Panel C; protein levels of phosphorylated PLB at residue Threonine 17 (Thr¹⁷); bars show sum of monomers and pentamers. Panel D; protein levels of phosphorylated PLB at residue Serine 16 (Ser¹⁶); bars show sum of monomers and pentamers. Panel E; protein levels of Ca^{2+} /calmodulin dependent kinase II (CaMKII δ), phosphorylated at residue Threonine 287 (Thr²⁸⁷) and non-phosphorylated (data not shown with bars). Protein expressions are from cardiomyocyte SR extracts (SERCA-2a and PLB) or cytosolic extracts (CaMKII δ) from 3 trained (TR) and 3 sedentary (SED) mice,

quantification in arbitrary units (mean $\pm$ SD). Significant difference between TR vs. SED; *: $p<0.01$.

Figure 3.

Panel A; fractional shortening. Panel B; intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) amplitude. Panel C; index of systolic Ca^{2+} sensitivity of myofilaments, corrected for diastolic shortening. Panel D; index of diastolic Ca^{2+} sensitivity of myofilaments. Panels A-D show data recorded at 5 Hz electrical field stimulation in cardiomyocytes from trained (TR, n=6, 5-8 cells/mouse) vs. sedentary (SED, n=6, 5-8 cells/mouse) mice, and after incubation with AIP; a specific inhibitor of Ca^{2+} /calmodulin dependent kinase II (CaMKII; n=6/group, 5-8 cells/mouse); see methods. Panel E; time to 50% re-lengthening, and Panel F: time to 50% Ca^{2+} transient decay in cardiomyocytes electrically stimulated 0.5-5 Hz from TR vs. SED mice, including after incubation with AIP (n=6/group, 5-8 cells/mouse with and without AIP). Panels E and F illustrate frequency-dependent acceleration of relaxation (FDAR). Data are presented as means $\pm$ SD. Significant difference between TR vs. SED; *: $p<0.01$, difference between TR vs. TR AIP; #: $p<0.01$, difference between TR AIP vs. SED AIP; Ψ : $p<0.05$. Ω in Panels E and F refers to faster relaxation (Panel E) and Ca^{2+} transient decay (Panel F) at the given stimulation frequency vs. the previous frequency in both TR and SED; hence, 5 Hz vs. 2 Hz, and 2 Hz vs. 0.5 Hz.