Exercise training increases the Ca\(^{2+}\) sensitivity of tension in rat cardiac myocytes

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Diffee, Gary M., Eric A. Seversen, and Marci M. Titus. Exercise training increases the Ca\(^{2+}\) sensitivity of tension in rat cardiac myocytes. *J Appl Physiol* 91: 309–315, 2001.—The heart is known to respond to a program of chronic exercise in ways that enhance cardiac function. However, the cellular mechanisms involved in training-induced improvements in the contractile function of the myocardium are not known. In this study we tested the hypothesis that increased contractility of the myocardium associated with exercise training is due, in part, to increases in the Ca\(^{2+}\) sensitivity of steady-state tension. Female Sprague-Dawley rats were randomly divided into sedentary control (C) and exercise-trained (T) groups. The T rats underwent 11 wk of progressive treadmill exercise (1 h/day, 5 days/wk, 26 m/min, 20% grade). Evidence of training effect included a 5.9% increase in heart mass, increases in heart weight-to-body weight ratio, and a 60% increase in skeletal muscle citrate synthase activity in T rats compared with C rats. After the training program, cardiac myocytes were isolated from T and C hearts. Myocytes were chemically skinned (i.e., the sarcolemma was removed) and attached to a force transducer, and steady-state tension was determined in solutions of various Ca\(^{2+}\) concentrations (\([\text{Ca}^{2+}]\)). Myocytes isolated from the hearts of T rats showed a significantly (P < 0.01) increased sensitivity of tension to \([\text{Ca}^{2+}]\). The \([\text{Ca}^{2+}]\) giving 50% of maximal tension (pCa\(_{50}\)) was 5.90 ± 0.033 and 5.82 ± 0.023 (SD) in T and C myocytes, respectively (n = 70 myocytes/group). This result suggests that exercise training affects the myofibrillar proteins, such that Ca\(^{2+}\) sensitivity is increased, and that this may be the mechanism that underlies, at least in part, the effect of training to increase myocardial contractility.

CHRONIC ENDURANCE EXERCISE training has been shown to elicit positive adaptations in the cardiovascular system that result in improved performance at maximal and submaximal work levels. Among these adaptations is a training-induced increase in peak left ventricular pressure development (5, 10, 27, 28). Because pressure development in the ventricle is driven by the capacity of the myocardium to generate tension or force, much work has been focused on the effect of exercise training on myocardial force generation. A number of earlier studies using intact ventricular muscle preparations showed that exercise training results in an increase in intrinsic isometric tension production in myocardial tissue, even when corrected for changes in muscle mass (19, 35, 37). Although this training-induced increase in myocardial contractility is widely accepted (see Ref. 20 for review), it is not universally seen (23, 29), and the cellular basis for this adaptation is not yet understood.

One hypothesis that has been investigated is that the increase in tension is brought about by an increase in the level of intracellular Ca\(^{2+}\) during activation. Previous work has examined the effect of training in a number of the “Ca\(^{2+}\)-handling” processes of myocardial cells. Evidence has been presented suggesting that exercise training alters sarcoplasmic Ca\(^{2+}\) influx (4, 35, 37), providing a greater level of activating Ca\(^{2+}\) in trained hearts. Training-induced alterations in sarcolemmal Na\(^+\)/Ca\(^{2+}\) exchange activity have been suggested in some studies (12, 36), although they have not been found in others (13). Changes in Ca\(^{2+}\) binding and transport by the sarcoplasmic reticulum (SR) have been reported in some training studies (24, 25) but have not been observed in others (13, 34). Experiments using single, electrically stimulated myocytes have not demonstrated an increase in the height of the intracellular Ca\(^{2+}\) transient during activation, especially as the extracellular Ca\(^{2+}\) concentration (\([\text{Ca}^{2+}]\)) bathing the cells approaches physiological levels (14, 21). Thus the ultimate effect of training on Ca\(^{2+}\) levels inside the cell is not clear.

An alternative hypothesis for the mechanism underlying increased contractile force in the myocardium is that exercise training may result in an increase in the sensitivity of the myofilaments to activation by Ca\(^{2+}\). An increase in Ca\(^{2+}\) sensitivity of tension would result in a greater level of isometric tension generation at the same intracellular [Ca\(^{2+}\)], an effect that is entirely consistent with reported effects of training on isometric tension in myocardial tissue. A training-induced increase in the Ca\(^{2+}\) sensitivity of the myofilaments would also be consistent with results indicating that in trained myocardium the contractile response is more sensitive to intracellular (21) and extracellular (12, 35) Ca\(^{2+}\). Under pathological conditions in which cardiac contractile function is diminished (myocardial infarc-
tion or chronic heart failure), the Ca\(^{2+}\) sensitivity of tension has been shown to be altered (15, 38). In an earlier study (35), there was no effect of exercise training on the Ca\(^{2+}\) sensitivity of myofibrillar ATPase activity in solution, but there is no direct information regarding the effect of training on the Ca\(^{2+}\) sensitivity of tension in a preparation with greater structural organization. Thus, in the present study, we tested the hypothesis that exercise training increases the Ca\(^{2+}\) sensitivity of tension development in cardiac myocytes.

To directly determine the Ca\(^{2+}\) sensitivity of the myocyte in solution, we measured tension development as a function of [Ca\(^{2+}\)] in myocytes from which the sarcolemma was chemically removed (i.e., “skinned” myocytes). We compared tension measurements in myocytes isolated from the hearts of sedentary control rats and from the hearts of rats subjected to an endurance treadmill training program.

**METHODS**

**Exercise training protocol.** Female Sprague-Dawley rats were randomly divided into exercise-trained (T) and sedentary control (C) groups (n = 10 animals/group). Rats in the T group were trained using an 11-wk treadmill training protocol that has previously been used to induce cardiac adaptations in the rat (8). The rats were trained on a rodent treadmill initially for 15 min/day at a speed of 13 m/min and at a 10% grade. The intensity and duration of the training were increased until at 5 wk the animals were running for 1 h/day at 26 m/min and a 20% grade. This level of intensity and duration were maintained throughout the remainder of the 11-wk training program. T and C animals were housed in a temperature- and light-controlled room (12:12-h light-dark cycle), and food and water were provided ad libitum. This protocol received approval from the University of Wisconsin-Madison Animal Use and Care Committee. Rats were weighed before beginning the training program and immediately before death.

**Cardiac myocyte preparation.** For contractile measurements, single myocyte-sized preparations were obtained by mechanical disruption of rat hearts by modification of a previously described method (11). Rats were anesthetized by inhalation of methoxyflurane, and the hearts were quickly excised. The heart was weighed and then placed in ice-cold Ca\(^{2+}\)-free relaxing solution and cut into four sections. The pieces were quick-frozen in liquid nitrogen and stored at –80°C. Myocytes for contractile measurements were prepared from this frozen tissue. In pilot data, there were no differences in contractile properties between cells prepared from frozen hearts and cells prepared from fresh hearts (data not shown). On the day of an experiment, a section of heart was removed from the freezer, placed in ~30 ml of cold relaxing solution, and minced with scissors. The tissue was further disrupted using a Waring blender on medium setting for 5 s. The resulting suspension of intact myocytes, groups of myocytes, and cell fragments was centrifuged for 90 s at 165 g. The myocytes were subsequently skinned by resuspending the pellet in relaxing solution plus 0.3% ultrapure Triton X-100 (Pierce Chemical) for 4 min. The pellet was then washed twice with cold relaxing solution. The remaining pellet was resuspended in 10 ml of relaxing solution and then kept on ice during the day of the experiment (18, 31, 32). Cells were stored overnight on ice at 4°C and remained viable for contractile measurements on the day after preparation. Cells were discarded after the 2nd day. There was no difference in contractile performance between 1st- and 2nd-day cells (data not shown). Cells were simultaneously prepared from trained and control heart tissue each time myocytes were prepared. Contractile measurements were made on an equal number of trained and control cells in each day (1–2 of each group/day) to minimize any day-to-day variations in pCa solutions. Experimenters were blinded regarding whether a given cell was from a C or T heart.

**Experimental apparatus.** The experimental technique for performing contractile measurements in cardiac myocytes is modified from one used previously to measure tension on skinned skeletal muscle fibers (3) and previously described for rat cardiac myocytes (18). Briefly, a drop of skinned myocytes and myocyte fragments suspended in relaxing solution was pipetted onto a coverslip placed on the mounting apparatus. Under the dissecting microscope, a myocyte-sized preparation that was rod shaped and had a clear striation pattern was selected for mounting. The myocyte preparation was attached between a capacitance-gauge transducer (sensitivity 20 mV/mg, resonant frequency 600 Hz; model 403, Cambridge Technology, Cambridge, MA) and a direct-current torque motor (model 308, Aurora Scientific). Myocytes were attached by placing the ends of the preparation into stainless steel troughs and secured by overlaying a 0.5-mm length of 4-0 monofilament nylon suture on each end and then tying the suture into the troughs with two loops of 10-0 monofilament suture. Myocyte preparations 150–200 μm long were used, since this provides enough cell length to grasp the ends with forceps for placement and proper alignment in the troughs. This preparation yields very-low-end compliance and highly uniform striation patterns during Ca\(^{2+}\) activations (18) (Fig. 1). The length of the preparation was adjusted so that sarcomere length was set to 2.35–2.40 μm in relaxing solution, and sarcomere length was monitored in pCa 4.5 to determine that it did not change significantly during activation.

Length changes during contractile measurements were introduced at one end of the preparation driven by voltage commands from a personal computer via a 16-bit digital-to-analog converter. Force and length signals were digitized at 1 kHz using a 16-bit analog-to-digital converter and were displayed and stored on a personal computer using custom software in LabView for Windows (National Instruments). The experimental chamber contained three troughs into which the myocyte was moved to effect rapid solution changes. The apparatus was cooled to 15°C using Peltier
devices (Cambion, Cambridge, MA) and a circulating water bath. The entire mechanical apparatus was mounted on a pneumatic vibration isolation table having a cutoff frequency of 1 Hz.

**Solutions.** Relaxing and activating solutions for skinned myocyte preparations have been described previously (3) and contain 7 mM EGTA, 1 mM free Mg$^{2+}$, 20 mM imidazole, 4 mM ATP, 14.5 mM creatine phosphate, pH 7.0 (at 15°C), 10$^{-9}$ M (relaxing solution) to 10$^{-4.5}$ M (maximally activating solution) free Ca$^{2+}$, and sufficient KCl to adjust ionic strength to 180 mM. The final concentrations of each metal, ligand, and metal-ligand complex were determined from the computer program of Fabiato (7).

**Tension-pCa relationships.** Tension was measured as a function of pCa (negative logarithm of [Ca$^{2+}$]) in the range of 9.0 to 4.5, as previously described for skinned cardiac myocytes (11). All experiments were carried out at 15°C. Tension was first measured in pCa 4.5 and then in randomly selected submaximal pCa solutions, with every fourth activation made in pCa 4.5 to assess any decline in myocyte performance. If maximum force (in pCa 4.5) declined by >20% from the first activation to the last, the cell was discarded and data from that cell were not used (18, 31, 32). For each activation, steady tension was allowed to develop; then the cell was slackened and subsequently transferred to relaxing solution (Fig. 2). Total tension was measured as the difference between steady developed tension and the tension baseline immediately after the slack step. Active tension was calculated by subtracting resting tension at pCa 9.0 from total tension. Tension at each pCa was expressed as a fraction of the maximum tension (pCa 4.5) obtained for that cell under the same conditions. As described by Hofmann et al. (11), data were analyzed by least-squares regression using the Hill equation

$$\log \left( \frac{P_{rel}}{1 - P_{rel}} \right) = n \log [Ca^{2+}] + k$$

where $P_{rel}$ is tension expressed as a fraction of maximal tension, $n$ is the Hill coefficient, and $k$ is the intercept of the fitted line with the x-axis, which corresponds to the [Ca$^{2+}$] at half-maximal tension (pCa$_{50}$). With the use of constants derived from the Hill equation, tension data were fit by computer with the following equation

$$P_{rel} = \frac{[Ca^{2+}]^n}{[Ca^{2+}]^n + [Ca^{2+}]^n}$$

**Citrate synthase assay.** Plantaris muscles were removed immediately after excision of the heart, trimmed of connective tissue, quick-frozen in liquid nitrogen, and stored at −80°C. The plantaris was thawed and homogenized in potassium phosphate buffer (pH 7.4) and assayed for citrate synthase activity at 25°C as previously described (30).

**Statistical analysis.** Between-group comparisons (C vs. T) were made using one-way ANOVA with post hoc analysis. $P < 0.05$ was considered to indicate a statistically significant difference between groups. For demonstration of the effect of training on submaximal tension, Fig. 3 was constructed by comparing pooled data from all the myocytes in each group (C vs. T). However, for statistical analysis, multiple cells from the same animal were considered as one measurement. Thus the mean pCa$_{50}$ was determined from seven cells per animal, and these data were averaged for statistical analysis of the effect of training on Ca$^{2+}$ sensitivity of tension (see Table 3).

**Fig. 2.** Force traces from representative control and trained myocytes. Myocytes were transferred to the indicated pCa solutions, and force was allowed to develop to a plateau. The cell was then slackened and transferred to relaxing solution. Total tension was measured as the difference between steady developed tension and the tension baseline immediately after the slack step.

**Fig. 3.** Relationship between relative tension and pCa in skinned myocytes. Data were compiled from 70 control myocytes and 70 trained myocytes. Relative tension data at each pCa were averaged from all myocytes in the group and are presented as means ± SD. Data were fitted by computer. Trained myocytes exhibited a leftward shift in the tension-pCa relationship compared with control myocytes. The pCa$_{50}$ of these composite curves was 5.83 for control and 5.89 for trained myocytes.
Table 1. Effect of exercise training program on rat heart and skeletal muscle characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>277.9 ± 14.94</td>
<td>267.3 ± 16.99</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>0.817 ± 0.043</td>
<td>0.865 ± 0.072</td>
</tr>
<tr>
<td>Heart wt/body wt, ×1,000</td>
<td>2.9 ± 0.13</td>
<td>3.24 ± 0.21</td>
</tr>
<tr>
<td>Plantaris citrate synthase activity, µmol·min⁻¹·g wet wt⁻¹</td>
<td>16.21 ± 2.38</td>
<td>25.87 ± 4.52</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different from control (P < 0.05).

RESULTS

As shown in Table 1, training did not have a significant effect on body weight, inasmuch as there was no significant difference in body weight between rats in the C and T groups after the treadmill training program. However, training elicited a 5.9% increase in absolute heart weight and an 11.7% increase in the heart weight-to-body weight ratio. Skeletal muscle (plantaris) citrate synthase activity was 60% higher in T than in C rats.

Figure 1 shows a typical myocyte mounted in the experimental apparatus. Tension measurements were made on seven myocytes from each animal in the T and C groups for a total of 140 myocytes tested. Table 2 gives mean data for all the cells from C and T hearts. Figure 3 was constructed by plotting, for each myocyte from C and T hearts (n = 70 each), the between-cell variation. The pCa50 for the T group gives mean data for all the cells from C and T hearts.

The pCa50, which refers to the [Ca²⁺] producing half-maximal tension, is a measure of the Ca²⁺ sensitivity of tension. Table 3 gives the pCa50 values for the myocytes from each animal in T and C groups. The pCa50 values for each animal were then averaged within each group to give the group (C vs. T) mean.

Table 2. Characteristics for single cardiac myocytes isolated from control and trained hearts

<table>
<thead>
<tr>
<th></th>
<th>Cell Length, µm</th>
<th>Sarcomere Length, µm</th>
<th>Cell Width, µm</th>
<th>Passive Force, µN</th>
<th>Maximal Force, kN/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>153 ± 25</td>
<td>2.35 ± 0.06</td>
<td>24 ± 7</td>
<td>0.77 ± 0.21</td>
<td>8.6 ± 1.9</td>
</tr>
<tr>
<td>Trained</td>
<td>162 ± 28</td>
<td>2.37 ± 0.08</td>
<td>26 ± 5</td>
<td>0.83 ± 0.27</td>
<td>9.1 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 70. Passive force is force measured in pCa 9.0 solution; maximal force is force measured in pCa 4.5 solution.

Table 3. Ca²⁺ sensitivity of tension in myocytes from trained and control animals

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>pCa50</th>
<th>Animal No.</th>
<th>pCa50</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>5.804 ± 0.065</td>
<td>T1</td>
<td>5.870 ± 0.059</td>
</tr>
<tr>
<td>C2</td>
<td>5.807 ± 0.119</td>
<td>T2</td>
<td>5.888 ± 0.064</td>
</tr>
<tr>
<td>C3</td>
<td>5.831 ± 0.074</td>
<td>T3</td>
<td>5.957 ± 0.125</td>
</tr>
<tr>
<td>C4</td>
<td>5.845 ± 0.064</td>
<td>T4</td>
<td>5.941 ± 0.082</td>
</tr>
<tr>
<td>C5</td>
<td>5.842 ± 0.059</td>
<td>T5</td>
<td>5.882 ± 0.099</td>
</tr>
<tr>
<td>C6</td>
<td>5.830 ± 0.031</td>
<td>T6</td>
<td>5.938 ± 0.067</td>
</tr>
<tr>
<td>C7</td>
<td>5.854 ± 0.051</td>
<td>T7</td>
<td>5.888 ± 0.049</td>
</tr>
<tr>
<td>C8</td>
<td>5.830 ± 0.104</td>
<td>T8</td>
<td>5.875 ± 0.047</td>
</tr>
<tr>
<td>C9</td>
<td>5.815 ± 0.381</td>
<td>T9</td>
<td>5.880 ± 0.074</td>
</tr>
<tr>
<td>C10</td>
<td>5.778 ± 0.110</td>
<td>T10</td>
<td>5.878 ± 0.063</td>
</tr>
<tr>
<td>Group Mean</td>
<td>5.824 ± 0.023</td>
<td></td>
<td>5.90 ± 0.053</td>
</tr>
</tbody>
</table>

Values for individual control (C1–C10) and trained (T1–T10) animals are means ± SD of 7 observations. Group mean is the average of the pCa50 values for each animal in the group. The SD of the group mean is a measure of the animal-to-animal variation. *Significantly different from control (P < 0.01).

DISCUSSION

The treadmill training protocol used in this study elicited a significant increase in plantaris muscle citrate synthase activity. This skeletal muscle adaptation, along with the effect of training on heart weight and heart weight-to-body weight ratio, is consistent with results of previous studies using this or a similar treadmill training protocol. These changes are indicative of the presence of an endurance training effect as a result of this training paradigm. This training protocol and substantially similar training protocols have been shown to increase maximal O₂ uptake, reduce resting and submaximal heart rate, increase peak left ventricular pressure during submaximal exercise (8), increase maximal isometric tension in papillary muscles (35), and increase the extent of shortening in intact isolated myocytes (21).
The primary finding of the present study is that myocytes isolated from exercise-trained hearts exhibit an increased Ca^{2+} sensitivity of tension evidenced by a leftward shift in the tension-pCa relationship. Thus more tension is developed at submaximal [Ca^{2+}]. This shift in Ca^{2+} sensitivity may underlie, in part, the training-induced increase in isometric tension that has been demonstrated in myocardial preparations (35) and the increase in cell shortening observed in single myocytes (21). Previous work examining the cellular basis underlying the increase in myocardial contractility associated with training has focused on training-induced alterations in processes that may increase intracellular [Ca^{2+}] during activation. Results of this work have not been conclusive. Although studies have suggested that sarcolemmal Ca^{2+} binding and transport may be altered by training (9, 35, 37), there has not been a direct measure of training-induced changes in Ca^{2+} influx. Some studies have reported altered sarcolemmal Na^{+}/Ca^{2+} exchange activity with training (12, 36), but this has not been observed in all studies (13). Changes in Ca^{2+} binding and transport by the SR have been observed in rats trained by swimming (24, 25), but no changes in SR function were found in rats trained by running (34). Direct measurements of intracellular Ca^{2+} levels during contraction have failed to show an increase in [Ca^{2+}] as a result of training (13, 19). The results of the present study indicate that tension development may be increased in the trained myocardium, even in the absence of changes in intracellular [Ca^{2+}].

Although the present study is the first direct study of Ca^{2+} sensitivity of tension in relation to exercise training, several studies have indirectly suggested a change in Ca^{2+} sensitivity of the myofibrillar apparatus. The contractile response has been shown to be more sensitive to extracellular Ca^{2+} in trained than in control myocardium (12, 35). Tibbits et al. (35) showed no effect of training on the Ca^{2+} sensitivity of myofibrillar ATPase activity in purified myofibrils in solution, and this was taken to indicate no effect of training on the Ca^{2+} sensitivity of the myofilaments. However, in a preparation with greater structural organization, intact myocytes isolated from trained hearts exhibited the same extent of shortening as control cells under conditions in which the activator [Ca^{2+}] was observed to be lower in the myocytes from trained animals (21). This result provided indirect evidence that the sensitivity of the contractile element to activation by Ca^{2+} was increased by exercise training.

Because in vivo much of the cardiac twitch contraction appears to occur at submaximal [Ca^{2+}] (6), the sensitivity of the myofilaments to activation by Ca^{2+} is a key component in regulating the force-generating ability of the myocardial cell. Figure 4 emphasizes the effect of the magnitude of shift in Ca^{2+} sensitivity reported in the present study on submaximal tension, illustrating the physiological significance of this shift. At these submaximal [Ca^{2+}] values, substantially more tension is developed by the trained than by the control myocytes.

One concern in interpreting single-cell physiological measurements is the extent to which sampled cells are representative of all myocytes within the myocardium. Our data shown in Table 3 show that the cell-to-cell variability in pCa_{50} is relatively low, on the same order of magnitude as the variability between hearts in a group. Thus it appears that there is not a great deal of heterogeneity among cells in trained hearts in how they respond to the training stimulus. It is thus likely that our sampled cells are representative of the myocardium. The between-cell variability we observed is similar to that in other studies measuring pCa_{50} in skinned myocytes (31, 32), and the between-heart variability compares favorably with variability in tension measurements in ventricular muscle preparations (35, 37).

The cellular or molecular mechanisms for the shift on Ca^{2+} sensitivity shown in the present study are not known. Alterations in contractile protein isoform content represent one potential mechanism that may confer altered contractile properties to the myocardium. Many of the myofibrillar proteins (myosin heavy and light chains, troponin I, C, and T, C-protein) exist as multiple isoforms. Recent studies suggest that regulatory protein content or isoform expression may be altered under pathological conditions involving decreased contractile function in the heart (1, 2, 17, 22, 38). However, little is known about the effect of exercise training on the protein content of the cardiac contractile element.

Phosphorylation of contractile proteins is a key mechanism by which mechanical properties are regulated in the heart. Cardiac troponin I and C-protein are phosphorylated by protein kinase A, resulting in alterations in cardiac contractile properties including shifts in the Ca^{2+} sensitivity of tension (32, 39). The regulatory light chain (RLC) of myosin is phosphorylated by a specific myosin light chain kinase. Phosphorylation of RLC increases the Ca^{2+} sensitivity of tension (33) and
thus represents a potential molecular mechanism to modulate myocardial contractile properties. Levels of RLC phosphorylation have been shown to be significantly increased in a heart failure model (16), but evidence of effects of exercise training on the level of RLC phosphorylation is conflicting. A significant relationship between exercise-induced improvements in cardiac function and increased RLC phosphorylation has been reported (26), but other studies have not found such a relationship (8). Experiments to examine the effect of training on cardiac contractile protein isoform content or phosphorylation state and the degree of correlation between any changes in contractile protein and changes in Ca$^{2+}$ sensitivity of tension are underway in our laboratory.

In summary, we have demonstrated that the Ca$^{2+}$ sensitivity of tension in rat myocardium is increased as a result of a treadmill training regimen. This increased sensitivity leads to greater steady-state tension during submaximal Ca$^{2+}$ activation of the myofilaments. It is likely that this adaptation at the level of the myofilaments underlies, at least in part, the improvement in myocardial contractility that has previously been seen with endurance exercise training.

REFERENCES


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