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Exercise training alters length dependence of contractile properties in rat myocardium

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Diffee, Gary M., and Daniel F. Nagle. Exercise training alters length dependence of contractile properties in rat myocardium. *J Appl Physiol* 94: 1137–1144, 2003. First published October 11, 2002; 10.1152/japplphysiol.00565.2002.—Myocardial function is enhanced by endurance exercise training, but the cellular mechanisms underlying this improved function remain unclear. Exercise training increases the sensitivity of rat cardiac myocytes to activation by Ca^{2+}, and this Ca^{2+} sensitivity has been shown to be highly dependent on sarcomere length. We tested the hypothesis that exercise training increases this length dependence in cardiac myocytes. Female Sprague-Dawley rats were divided into sedentary control (C) and exercise-trained (T) groups. The T rats underwent 11 wk of progressive treadmill exercise. Heart weight increased by 14% in T compared with C rats, and plantaris muscle citrate synthase activity showed a 39% increase with training. Steady-state tension was determined in permeabilized myocytes by using solutions of various Ca^{2+} concentration (pCa), and tension-pCa curves were generated at two different sarcomere lengths for each myocyte (1.9 and 2.3 μm). We found an increased sarcomere length dependence of both maximal tension and pCa_{50} (the Ca^{2+} concentration giving 50% of maximal tension) in T compared with C myocytes. The ΔpCa_{50} between the long and short sarcomere length was 0.084 ± 0.023 (mean ± SD) in myocytes from C hearts compared with 0.132 ± 0.014 in myocytes from T hearts (n = 50 myocytes per group). The Δmaximal tension was 5.11 ± 1.42 kN/m^2 in C myocytes and 9.01 ± 1.28 in T myocytes. We conclude that exercise training increases the length dependence of maximal and submaximal tension in cardiac myocytes, and this change may underlie, at least in part, training-induced enhancement of myocardial function.

cardiac myocytes; calcium sensitivity; endurance training; sarcomere length

CHRONIC ENDURANCE EXERCISE training has been shown to enhance the functional capacity of the heart as evidenced by greater maximal cardiac output and greater maximal and submaximal stroke volume (5, 9, 20, 35, 36). Stroke volume is subject to a number of influences, including end-diastolic volume, intrinsic contractile function of the myocardium, and the afterload placed on the heart. There is a substantial body of evidence suggesting that endurance exercise training has significant effects on end-diastolic volume (7, 17, 25, 36) and afterload (20), but there are also a number of studies that have demonstrated a training-induced enhancement of the intrinsic contractile function of the myocardium. These include studies of ventricular function in the intact heart (5, 14, 20) as well as studies of the contractile performance of myocardial muscle preparations or single cardiac cells (10, 28, 30, 31, 40, 41, 43). A number of studies have attempted isolate the cellular and/or molecular mechanism(s) for an enhancement of contractile function (6, 23, 33, 34, 40, 41, 43), but the results of these studies have yet to provide a clear picture of the effect of exercise training on various subcellular processes (reviewed in Ref. 29). Thus there is the need for further determination of cellular mechanisms for training-induced improvements in contractile function.

Our laboratory has previously shown (10) that exercise training has a direct effect on the contractile apparatus. We observed that exercise training increased the Ca^{2+} sensitivity of tension in rat skinned cardiac myocytes, resulting in greater tension at submaximal Ca^{2+} levels in myocytes from trained animals than in control myocytes at the same Ca^{2+} concentration ($[Ca^{2+}]$). The cellular or molecular basis for this increased Ca^{2+} sensitivity has not been determined, although there are a number of molecular factors that are known to affect Ca^{2+} sensitivity in the myocardium, including regulatory protein isoform changes and phosphorylation of contractile proteins (3, 39, 45, 46). In addition to these factors, it is known that Ca^{2+} sensitivity of tension in cardiac muscle is highly sensitive to changes in muscle length or sarcomere length, much more so than skeletal muscle (4, 11, 18, 22, 26, 27). As muscle cell length or sarcomere length is increased, the sensitivity of the contractile element to activation by Ca^{2+} has been shown to be increased. It is thought that this length dependence of Ca^{2+} sensitivity is an underlying mechanism of the Frank-Starling relationship, which describes the increase in stroke volume with increased end-diastolic volume. We hypothesized that our previously reported increase in Ca^{2+} sensitivity as a result of exercise training was due to a training-induced enhancement of the length dependence of this parameter. An earlier study (28)

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reported that exercise training resulted in a change in the length-tension relationship in rat papillary muscle such that, for a given increase in muscle length, tetanic tension increased more in muscles from trained hearts compared with muscle from control muscle. A more recent study on intact myocytes (31) found a similar effect of training on maximal tension. The effects of training on the relationship between length and submaximal tension has not been determined, but evidence indicates that in vivo much of the myocardial twitch contraction occurs at submaximal [Ca^{2+}] (12). Thus information regarding the activation of force at submaximal [Ca^{2+}] is crucial to understanding adaptation at the cellular level. In addition, there are a number of subcellular processes involved in force regulation that are affected by changes in muscle or sarcomere length (1, 2). Therefore, the purpose of this study was twofold: *1*) to determine whether the previously observed effect of exercise training on the length sensitivity of maximal tension (28, 31) was present at the cellular/myofilament level even after removal of extracellular and other elastic elements and *2*) to determine the effect of exercise training on the length dependence of Ca^{2+} sensitivity in cardiac myocytes. To study these questions, we used single "skinned" cardiac myocytes from which the membranes were removed to isolate the myofilaments from extracellular and other subcellular components. We measured maximal tension and the Ca^{2+} sensitivity of submaximal tension in the same myocyte at different sarcomere lengths to observe any shift in length dependence of tension properties.

METHODS

Exercise training protocol. Female Sprague-Dawley rats were randomly divided into a control group ($n = 8$) and a training group ($n = 8$). The animals were housed in individual cages on a 12:12-h light-dark cycle and had access to food and water ad libitum. The training consisted of an 11-wk treadmill training protocol that had been shown previously to increase maximal oxygen uptake and increase cardiac performance at the whole heart (14), myocardial (40, 41), and single-cell level (10). The rats were trained on a rodent treadmill starting at 15 min/day at a speed of 10 m/min and at a 10% grade. The intensity and duration of the training sessions were progressively increased until at *week 6* the animals were running at 26 m/min, up a 20% grade for 1 h/day. This intensity and duration were then maintained though the final 5 wk. This protocol received approval from the University of Wisconsin-Madison Animal Use and Care Committee.

Cardiac myocyte preparation. For contractile measurements, single myocyte-sized preparations were obtained by mechanical disruption of ventricular tissue as described previously (10). The animals were anesthetized by inhalation of methoxyflurane, and the hearts were quickly excised and weighed. The heart was placed in ice-cold Ca^{2+}-free relaxing solution; trimmed of atria, connective tissue, and vascular tissue; and cut into three sections. These sections were quick-frozen in liquid nitrogen and stored at −80°C until used to prepare myocytes for contractile measurements. On the day of an experiment, one heart section was placed into ~30 ml of ice-cold relaxing solution, minced with scissors, and further disrupted in a Waring blender. The resulting suspension of cells and cell fragments was centrifuged, and the pellet was then resuspended in cold relaxing solution plus 1% Triton X-100 for 7 min. The resulting skinned myocytes were resuspended in 8–10 ml of relaxing solution and kept on ice throughout the days of the experiments. All contractile experiments were performed within 48 h of cell preparation. Cells were discarded after the second day.

Experimental apparatus. The experimental apparatus has been described previously (10). Skinned cardiac myocyte preparations were attached between a capacitance-gauge transducer (model 403, Aurora Scientific) and a direct-current torque motor (model 308, Aurora Scientific) by placing the ends of the preparation into stainless steel troughs. The ends were then secured to the troughs by overlaying a ~0.5 mm length of 4-0 monofilament suture over each end and then tying the suture to the trough by use of a loop of 10-0 monofilament suture.

The experimental preparation was viewed by using an inverted microscope (Olympus IX50) with a ×20 objective and fitted with a ×15 black-and-white photoeyepiece (Sony CCD-IRIS). A video image of the myocyte was displayed on a monitor, and sarcomere length was measured by using a micrometer against this image (Fig. 1). Length changes during contractile measurements were 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 by 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 wells into which the myocyte could be moved to change bathing solutions. The experimental apparatus was cooled to 15°C by using a Peltier device (Cambion, Cambridge, MA) and a circulating water bath.

Solutions. Relaxing and activating solutions for skinned myocyte preparations 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); various free [Ca^{2+}] between 10^{-9} and $10^{-4.5}$ M (maximally activating solution); and sufficient KCl to adjust ionic strength to 180 mM. The final concentrations of each metal, ligand, and metal-ligand complex are determined from the computer program of Fabiato (13).

Tension-pCa relationships. Tension was measured as a function of pCa (−log [Ca^{2+}]) in the pCa range of 9.0–4.5 as previously described (10). Tension was measured first in pCa 4.5 solution and then in randomly ordered submaximal pCa solutions. Every third activation was made in pCa 4.5 to assess any decline in myocyte performance. For each activation, steady tension was allowed to develop, at which point the cell was slacked by 20% of its initial length and transferred to pCa 9.0 solution. Total tension was calculated as the difference between steady-state tension and the tension baseline immediately after the preparation was slacked (Fig. 2). Active tension was determined by subtracting passive tension in pCa 9.0 from total tension. Maximal tension (in pCa 4.5 solution) was determined and normalized for the cross-sectional area of the cell. Cross-sectional area was calculated from the diameter of the cell by assuming a circular cross section. Tension at each pCa was expressed as a percentage of maximal tension for that preparation. Data were analyzed by least squares regression using the Hill equation as described previously (10). Analysis using the Hill equation yielded values for the pCa_{50} (the pCa giving half-maximal tension), which was used as an index of Ca^{2+} sensitivity of tension. To determine the length dependence of tension properties, tension-pCa measurements were carried out two

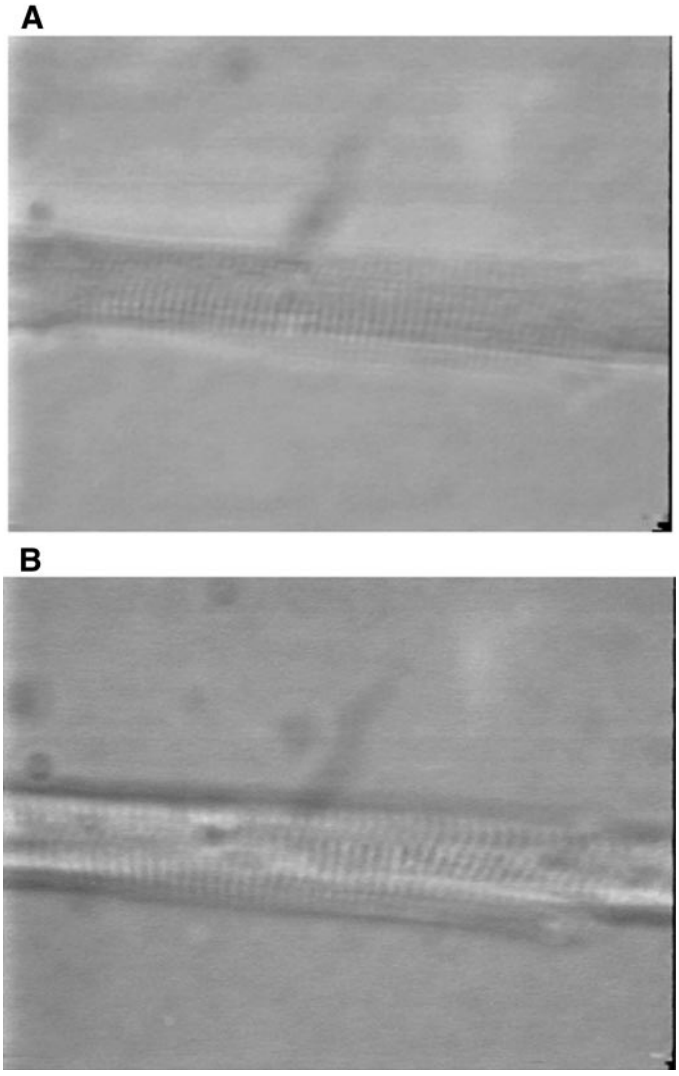

Fig. 1. Photomicrograph of a representative myocyte attached to the experimental apparatus. *A*: a myocyte in relaxing solution (pCa 9.0) set to a sarcomere length of 1.9 μm. *B*: the same myocyte stretched to a sarcomere length of 2.3 μm. Sarcomere length was measured as described in METHODS.

times for each cell: once with the myocyte preparation set to a sarcomere length of 1.9 μm and again with the sarcomere length set to 2.3 μm. These sarcomere lengths were chosen because they likely represent the working range of sarcomere lengths in the intact myocardium (32) and because these sarcomere lengths have been used previously in studies of length dependence of tension (4, 26, 27). Sarcomere lengths were monitored during activation, and if sarcomere length shortened significantly during activation or if the sarcomere pattern became disorganized, the preparation was discarded. The order of sarcomere length settings was randomized. The experimenter was also blinded as to whether a given cell was from a trained or control animal.

Citrate synthase. Plantaris muscles were removed after excision of the heart, trimmed of connective tissue, quick-frozen in liquid nitrogen, and stored at −80°C. The plantaris muscles were thawed and homogenized in potassium phosphate buffer (pH 7.4) and assayed for citrate synthase activity at 25°C as previously described (37).

Statistical analysis. Comparisons between data from trained and control groups were made by using a one-way ANOVA with post hoc analysis, with $P < 0.05$ used to indicate a significant difference. Figure 3 was constructed by comparing pooled tension data from each of the myocytes in the trained and control groups at each of the two sarcomere lengths (1.9 and 2.3 μm). However, to determine between-group differences in the length dependence of maximal and submaximal tension, the difference in maximal tension (Δmax tension) and in pCa_{50} (ΔpCa_{50}) accompanying the change in sarcomere length was determined for each cell individually. These data were pooled for a minimum of six cells from each animal, producing a mean Δmax tension and a mean ΔpCa_{50} for each animal (see Table 3). These were then pooled to produce a mean Δmax tension and a mean ΔpCa_{50} for the trained ($n = 8$) and control ($n = 8$) groups. Trained and control data were compared by using a Student's *t*-test. A level of $P < 0.05$ was used to indicate significance.

RESULTS

The treadmill training program used in this study elicited typical training effects in the rats as shown in Table 1. There was no significant difference in body weight between trained and control rats either before or after the 11-wk treadmill training program. However, training did elicit a 14% increase in absolute heart weight and a 14.4% increase in the heart weight-to-body weight ratio. In addition, the plantaris muscles taken from the trained animals showed a 39% higher citrate synthase activity compared with control plantaris muscle.

Figure 1 shows a typical myocyte preparation mounted in the experimental apparatus and set to the two different sarcomere lengths used in the experiment. Table 2 shows characteristics for the myocytes from the trained and control groups at the two different sarcomere lengths. These data give the pooled values for all myocytes studied in each group ($n = 50$ per group) irrespective of which animal the myocytes were from.

Figure 2*A* shows raw tension data at different $[Ca^{2+}]$ (expressed as pCa) and at the two different sarcomere lengths. Tension at each pCa was measured as the difference between tension just before the slack step minus tension just after the step. Figure 2*B* shows tension-pCa curves at the two different sarcomere lengths in representative myocytes from trained and control animals. These curves illustrate the leftward shift in the tension pCa curves observed when the myocyte preparation is stretched from a shorter to a longer sarcomere length.

We used two different methods to calculate the effect of training on the length dependence of maximal tension, pCa_{50}, and the Hill coefficient. The first is shown in Fig. 3, which shows composite tension-pCa curves from control (*A*) and trained (*B*) myocyte preparations. Figure 3 was constructed by plotting, for each pCa value, the mean of the pooled tension values from each myocyte from control or trained animals ($n = 50$ each) for that pCa solution at each sarcomere length. Tension was always expressed as a fraction of the maximum tension for that cell. As Fig. 3 shows, the effect of

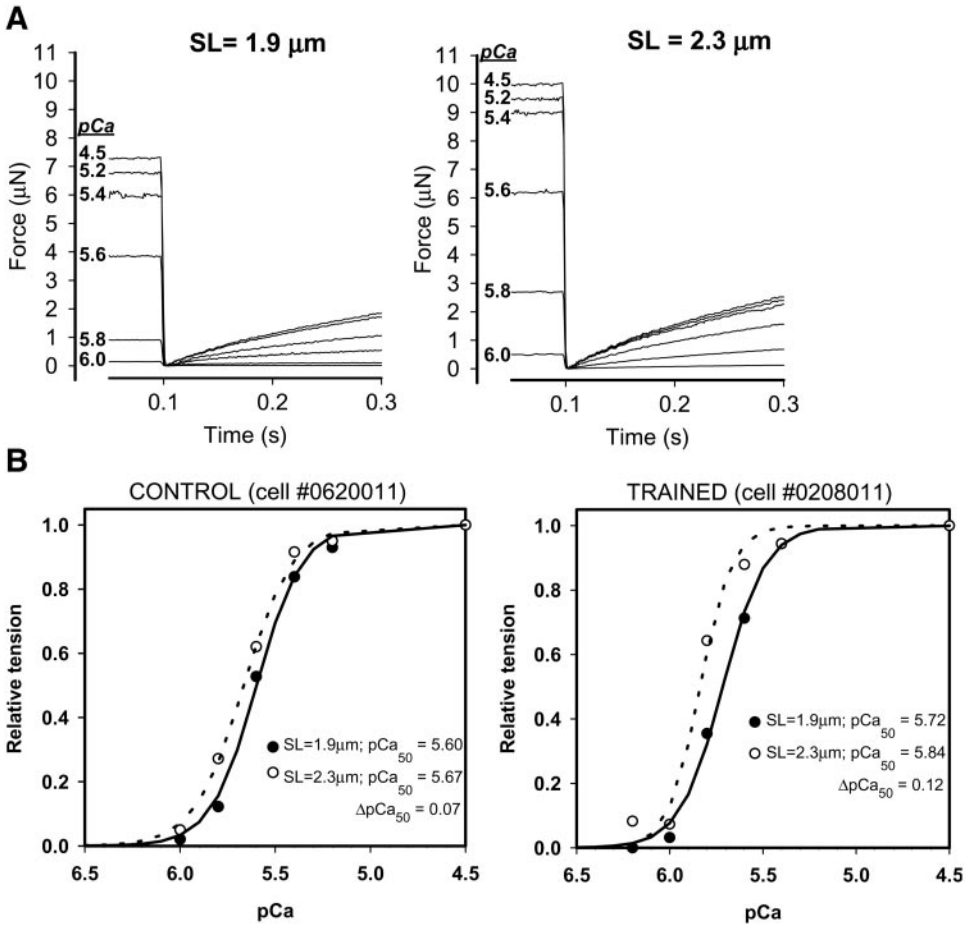

Fig. 2. *A*: force traces from a representative myocyte. Myocytes were moved into the indicated pCa solutions, and force was allowed to develop to a plateau at which point the cell was slackened and transferred back to relaxing solution. Total tension was measured as the difference between steady developed tension and the tension immediately after the slack. This process was completed for the full range of pCa solutions with the cell set to 1 sarcomere length (SL) and then repeated at the other sarcomere length. *B*: force measured at each pCa was then plotted as a function of pCa, resulting in a tension-pCa curve for each cell at each sarcomere length. Tension-pCa curves are shown for representative myocytes from trained and control hearts. The difference in the pCa giving half-maximal tension (pCa_{50}) at each sarcomere length was calculated, and this ΔpCa_{50} was used as the measure of the length dependence of Ca^{2+} sensitivity for each cell.

changing sarcomere length was much greater in myocytes from trained hearts compared with control. Although increasing sarcomere length from 1.9 to 2.3 μm induced a leftward shift in the tension-pCa relationship (i.e., increased the tension at any given pCa), this effect was much greater in the myocytes from trained hearts. The pCa_{50} is a convenient measure of Ca^{2+} sensitivity. The pCa_{50} for the control myocytes was 5.56 at sarcomere length of 1.9 μm and 5.65 at sarcomere length of 2.3 μm for a ΔpCa_{50} of 0.09. For myocytes from trained hearts, the pCa_{50} was 5.59 at sarcomere length of 1.9 μm and 5.72 at sarcomere length of 2.3 μm for a ΔpCa_{50} of 0.13. The Hill coefficient, which is a measure of the degree of cooperativity in tension development, was 3.43 and 3.75 in control myocytes and 3.42 and 3.68 in trained myocytes, at short and long lengths, respectively.

It has been suggested (8, 18) that this method, i.e., calculation of pCa_{50} and n from averaged tension values, leads to an underestimation of values of the Hill coefficient. Therefore we also measured maximal tension, pCa_{50} values, and n values for each myocyte at each of the two different sarcomere lengths as well as the difference (Δ) in these values between the two sarcomere lengths. These data were pooled with the data from the other myocytes for that animal to calculate means for each animal. Finally, the mean values for each animal were pooled into control group means and trained group means for each category. These data are all given in Table 3. We found that, for pCa_{50}, there was not a significant difference between trained and control groups at the shorter sarcomere length, but that mean pCa_{50} was significantly ($P < 0.05$) higher in the trained group compared with control at the longer sarcomere length. Maximal tension was not significantly different between trained and control at either sarcomere length. Exercise training increased the length dependence of both maximal tension and the Ca^{2+} sensitivity of submaximal tension because both Δmax tension and ΔpCa_{50} were significantly higher in the trained group compared with control. There was no effect of training or sarcomere length on the Hill coefficient.

DISCUSSION

Effects of exercise on length dependence of tension. The primary finding of this study is the effect of exercise training to increase the sarcomere length dependence of contractile properties such as maximal tension and Ca^{2+} sensitivity of submaximal tension in cardiac myocytes. We found that when myocytes were stretched from a sarcomere length of 1.9–2.3 μm (a sarcomere length range that likely encompasses the

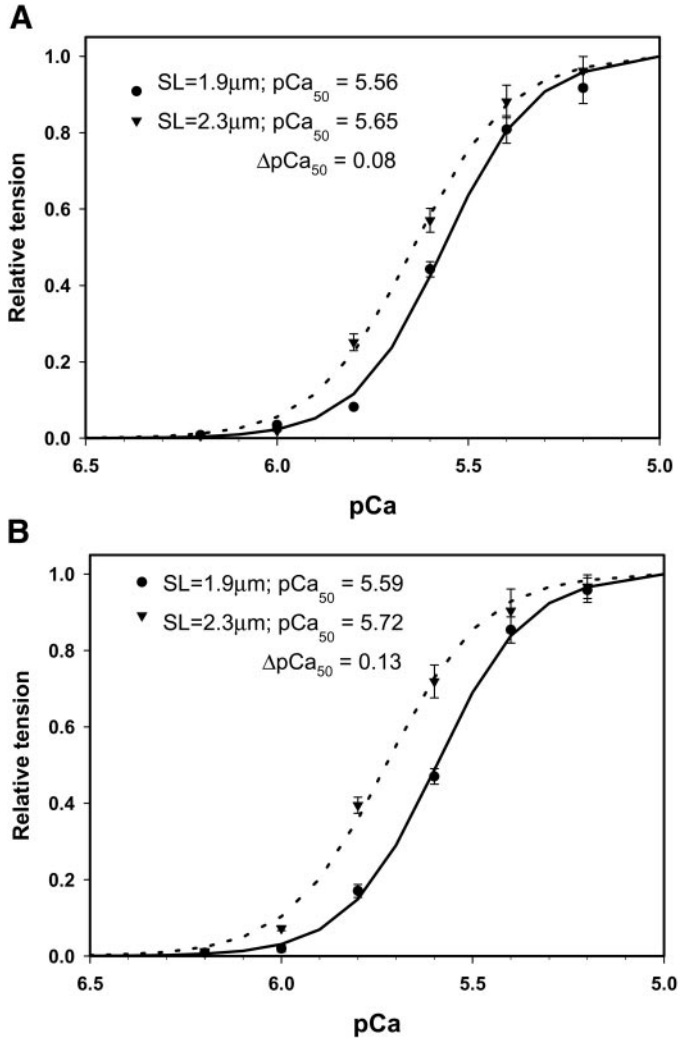

Fig. 3. Composite tension-pCa curve for control (*A*) and trained (*B*) myocytes. Data were compiled from 50 control and 50 trained myocytes. Relative tension data at each pCa were averaged at each sarcomere length from all myocytes in each group. Data points are presented as means ± SD. Lines are the best-fit regression line using the Hill equation as described in METHODS. pCa_{50} values are given for each sarcomere length for the 2 groups. Hill coefficients are 3.43 and 3.75 for control myocytes at short and long sarcomere lengths, respectively, and 3.42 and 3.68 for trained myocytes at short and long sarcomere lengths, respectively.

working range of the myocardium; Ref. 32), both maximal tension and the Ca^{2+} sensitivity of submaximal tension were increased, but they were increased to a significantly greater extent in myocytes isolated from trained hearts compared with control.

In an earlier study, Molé (28) had observed a steeper length-tension relationship in the papillary muscles from the hearts of exercised trained rats. This myocardial muscle preparation contains elastic elements both in series and in parallel with the contractile element. A recent study that used force measurements in intact, electrically stimulated myocytes also found that exercise training induced an increase in the slope of the relationship between sarcomere length and maximal force (31). Because it is known that changes in muscle length have effects on intracellular [Ca^{2+}] during a myocardial twitch (2), it is unclear from these earlier studies whether a training-induced increase in the length-tension relationship was due to adaptation at the level of the contractile element or other cellular or extracellular components. The results of the present study using skinned myocytes suggest that a training-induced increase in length dependence of maximal tension is a property of the contractile element itself.

Table 1. *Effect of exercise training on rat heart and skeletal muscle characteristics*

	Control	Trained
Body wt		
Pretraining, g	196.29 ± 1.67	194.38 ± 1.79
Posttraining, g	256.50 ± 4.31	256.13 ± 2.26
Heart wt, g	0.6765 ± 0.0152	0.7709 ± 0.0147*
Heart wt/body wt × 1,000	2.63 ± .042	3.01 ± .052*
Plantaris citrate synthase activity, μmol·min^{-1}·g wet wt^{-1}	16.76 ± .764	23.36 ± 1.365*

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

It is interesting to note that although our laboratory (10) and others (43) have previously described an effect of exercise training to increase the Ca^{2+} sensitivity of tension in cardiac myocytes, Natali et al. (31) recently reported no effect of training on Ca^{2+} sensitivity in intact myocytes. The results of the present study demonstrate that training increases the sensitivity of this property to differences in sarcomere length. Thus the effect of training on the Ca^{2+} sensitivity of tension is highly sensitive to the sarcomere length at which the measurements are done. As a consequence of the increased sarcomere length dependence, we found that trained myocytes again exhibited greater Ca^{2+} sensitivity of tension compared with control when measured at long sarcomere lengths, but this difference was not evident when measured at the short sarcomere length. The results of Natali et al. demonstrating no effect of training on Ca^{2+} sensitivity were obtained with myocytes at a short sarcomere length.

Implications for ventricular function. There are a number of studies that have reported an increase in end-diastolic volume with endurance exercise training, and this increase is widely thought to underlie, at least in part, the training-induced increase in stroke volume

Table 2. *Characteristics for single cardiac myocytes at two different sarcomere lengths*

	Cell Length, μm	Cell Width, μm	Passive Force, μN	Maximal Force, kN/m^2
Control				
SL = 1.9 μm	123 ± 33	28 ± 3.9	0.75 ± 0.26	11.4 ± 4.1
SL = 2.3 μm	167 ± 27	25 ± 4.3	0.81 ± 0.19	18.7 ± 6.3
Trained				
SL = 1.9 μm	131 ± 21	30 ± 4.6	0.79 ± 0.33	10.2 ± 5.8
SL = 2.3 μm	174 ± 31	28 ± 4.1	0.84 ± 0.24	20.3 ± 5.6

Values are means ± SD. SL, sarcomere length.

Table 3. *Maximal tension and pCa_{50} and Hill coefficient values in myocytes from trained and control animals at two different sarcomere lengths*

	SL = 1.9 μm			SL = 2.3 μm			1.9 vs. 2.3 μm		
Animal No.	pCa_{50}	n	Maximal tension, kN/m^2	pCa_{50}	n	Maximal tension, kN/m^2	ΔpCa_{50}	Δn	ΔMaximal tension, kN/m^2
C1	5.567 ± 0.010	3.73 ± 0.13	14.93 ± 4.82	5.635 ± 0.024	4.24 ± 0.43	20.34 ± 3.35	0.068 ± 0.019	0.51 ± 0.33	5.41 ± 1.45
C2	5.619 ± 0.039	3.22 ± 0.34	8.35 ± 3.76	5.679 ± 0.037	3.94 ± 0.26	12.40 ± 2.79	0.060 ± 0.025	0.72 ± 0.31	4.05 ± 1.23
C3	5.563 ± 0.027	3.67 ± 0.28	15.88 ± 5.22	5.626 ± 0.025	3.71 ± 0.39	19.37 ± 4.33	0.063 ± 0.017	0.04 ± 0.27	3.49 ± 0.66
C4	5.602 ± 0.050	2.95 ± 0.54	13.25 ± 4.39	5.673 ± 0.049	3.26 ± 0.42	17.73 ± 4.65	0.071 ± 0.023	−0.29 ± 0.41	4.48 ± 1.83
C5	5.545 ± 0.044	3.84 ± 0.27	7.96 ± 3.01	5.622 ± 0.060	3.25 ± 0.15	11.82 ± 3.81	0.077 ± 0.031	−0.59 ± 0.22	3.86 ± 1.27
C6	5.622 ± 0.033	3.06 ± 0.30	15.33 ± 4.68	5.732 ± 0.035	3.87 ± 0.33	21.21 ± 3.46	0.11 ± 0.013	0.81 ± 0.43	5.88 ± 1.79
C7	5.589 ± 0.030	3.47 ± 0.42	14.27 ± 3.50	5.707 ± 0.025	3.55 ± 0.21	20.29 ± 4.27	0.118 ± 0.020	0.08 ± 0.12	6.02 ± 1.59
C8	5.559 ± 0.037	3.35 ± 0.25	9.94 ± 2.88	5.661 ± 0.033	3.20 ± 0.28	17.68 ± 3.04	0.102 ± 0.012	0.77 ± 0.18	7.74 ± 2.59
Mean	5.583 ± 0.029	3.57 ± 0.38	12.49 ± 3.24	5.667 ± 0.039	3.83 ± 0.32	17.61 ± 3.61	0.084 ± 0.023	0.26 ± 0.53	5.11 ± 1.42
T1	5.557 ± 0.040	3.68 ± 0.45	13.91 ± 3.22	5.717 ± 0.038	3.54 ± 0.29	22.06 ± 4.75	0.160 ± 0.013	0.66 ± 0.32	8.15 ± 1.38
T2	5.617 ± 0.021	3.82 ± 0.27	8.78 ± 2.69	5.738 ± 0.026	3.33 ± 0.52	17.65 ± 4.30	0.121 ± 0.020	−0.09 ± 0.21	8.87 ± 1.11
T3	5.667 ± 0.029	3.41 ± 0.16	17.20 ± 3.85	5.791 ± 0.025	3.78 ± 0.34	27.23 ± 5.61	0.124 ± 0.025	0.37 ± 0.17	10.03 ± 3.79
T4	5.606 ± 0.030	4.11 ± 0.49	12.48 ± 4.24	5.735 ± 0.028	3.91 ± 0.46	21.04 ± 5.08	0.129 ± 0.011	−0.20 ± 0.19	8.56 ± 1.89
T5	5.618 ± 0.018	3.98 ± 0.52	9.90 ± 2.98	5.749 ± 0.017	3.77 ± 0.18	18.17 ± 3.89	0.131 ± 0.022	−0.21 ± 0.25	8.27 ± 2.17
T6	5.741 ± 0.028	3.71 ± 0.24	7.06 ± 2.74	5.856 ± 0.065	3.89 ± 0.36	16.35 ± 3.65	0.115 ± 0.061	0.18 ± 0.36	9.29 ± 1.43
T7	5.639 ± 0.037	3.22 ± 0.36	8.78 ± 3.42	5.769 ± 0.040	4.12 ± 0.10	20.29 ± 4.53	0.130 ± 0.012	0.90 ± 0.41	11.51 ± 2.85
T8	5.572 ± 0.023	3.39 ± 0.27	8.67 ± 3.86	5.719 ± 0.031	2.78 ± 0.31	16.07 ± 4.26	0.147 ± 0.010	0.41 ± 0.36	7.40 ± 1.21
Mean	5.627 ± 0.057	3.67 ± 0.31	10.85 ± 3.40	5.759 ± 0.046*	3.82 ± 0.39	19.86 ± 3.69	0.132 ± 0.014*	0.15 ± 0.46	9.01 ± 1.28*

Values for each animal are means ± SD from a minimum of 6 myocytes from each animal. Group means are averages ± SD of the values for each animal in the group. pCa_{50}, Ca^{2+} concentration giving 50% maximal tension; n, Hill coefficient. *Significantly different ($P < 0.05$) compared with control.

(7, 17, 25, 35, 36). One possible mechanism for a training-induced increase in end-diastolic volume is increased myocardial compliance, an effect that has been suggested in some studies (25, 44) but not in others (17). Changes in myocardial compliance might be expected to result in changes in passive tension properties in cardiac myocytes. We found no effect of exercise training on the length dependence of passive (resting) tension, an effect that is also consistent with an earlier result in a multicellular preparation (28) as well as a more recent study in intact myocytes (31). The latter study also determined that exercise training had no effect on the differential expression of titin isoforms in myocardial tissue, a likely determinant of passive tension in the myocardium (16).

The results of the present study regarding the effects of sarcomere length changes on active tension do suggest that the trained heart may be more sensitive to changes in ventricular volume. Thus training-induced enhancement of myocardial contractile performance would be expected to be most prominent at longer muscle lengths (corresponding to greater ventricular volumes). Indeed, a number of studies in the intact heart have observed that the effects of training on myocardial function are more pronounced at higher filling pressures or ventricular volumes (5, 35). Levine et al. (25) found a steeper relationship between stroke volume and filling pressure in endurance-trained athletes, an effect that they attributed to increased compliance but one that is also consistent with an increased length dependence of active tension properties. In contrast, Fuller and Nutter (15) measured stroke work across a range of filling pressures and found no change in the slope of this relationship with training, but they also found no other evidence for enhanced contractility as a result of their training model.

Comparison with previous studies of length dependence. A strength of the approach used in this study was that tension measurements were made at the different sarcomere lengths in the same myocyte. This allowed the calculation of Δmax tension and ΔpCa_{50} values within a single myocyte rather than relying on comparisons of absolute values of tension or pCa_{50} across different myocytes. There is substantial variability in absolute pCa_{50} values among different studies even under identical conditions. For example, absolute pCa_{50} values for control rat myocytes at sarcomere length ~2.3 and at 15°C have recently been reported as 5.83 (10), 5.65 (27), 5.71 (38), and 5.77 (19). Within the present study, pCa_{50} values at a single sarcomere length had a large range. Because of this variability, it seems unlikely that differences in the pCa_{50} values would have been observed had we attempted to compare pCa_{50} values from separate myocytes at different sarcomere lengths.

The ΔpCa_{50} values obtained in the present study are very similar to ΔpCa_{50} values obtained in other studies of sarcomere length dependence in skinned single myocytes. We observed a ΔpCa_{50} of 0.084 in control myocytes, which increased to 0.132 in trained myocytes. Previous reports of shifts in pCa_{50} with similar changes in sarcomere length include 0.09 in rat myocytes (27) and 0.12 in mouse myocytes (26). Measurements in skinned trabeculae preparations tend to show greater shifts in pCa_{50} with values including 0.11 pCa (4), 0.21 (18), 0.31 (22), and 0.14 (11). We found no effect of changing sarcomere length on the Hill coefficient, consistent with previous reports (11, 18, 22). We

also found no effect of exercise training on the Hill coefficient or its sensitivity to sarcomere length.

The value of same-cell comparisons is even more clear in measurements of maximal tension. Our measurements of maximal tension (normalized for cross-sectional area) showed tremendous variability, probably owing to error in the estimation of cross-sectional area on the basis of cell width. Thus we found no significant difference in maximal tension values between trained and control at either sarcomere length. However, using within-cell measurements to assess the effect of altered sarcomere length eliminates this source of variability, and we observed a significant effect of training to increase the length dependence of maximal tension, as measured by the Δmax tension values.

A disadvantage of the approach used in the present study is that it is not possible to make tension measurements at a larger number of sarcomere lengths. The large number of activations of the myocyte necessary to characterize Ca^{2+} sensitivity at each sarcomere length makes it unlikely that the cells would survive measurements at additional sarcomere lengths. Thus it was not possible to determine the slope of the relationship between sarcomere length and maximal or submaximal tension properties or the effect of exercise training on this slope. Kentish et al. (22) suggest that the relationship between force and sarcomere length is curvilinear at most [Ca^{2+}], whereas a recent study by Dobesh et al. (11) indicates that the relationship between Ca^{2+} sensitivity and sarcomere length is highly linear.

Possible mechanisms of increased length dependence. The mechanism by which exercise training increases the length dependence of maximal and submaximal tension is not clear. Two separate mechanisms have been proposed to underlie the length dependence of Ca^{2+} sensitivity. Babu et al. (4) proposed that cardiac troponin C (TnC) has some intrinsic length-sensitive properties that confer increased length dependence of Ca^{2+} sensitivity compared with skeletal muscle. They observed that when native cardiac TnC was stripped from trabeculae and replaced by skeletal troponin C (sTnC), the length dependence of Ca^{2+} sensitivity was decreased, although this effect was not seen when cardiac TnC was replaced by skeletal TnC in a transgenic mouse model (26). This suggests that differences in Ca^{2+} sensitivity seen in the present study might result from differences in myofilament protein expression. Although there is no indication of plasticity in the expression of cardiac vs. skeletal TnC isoforms, shifts in the expression of other myofilament proteins may be possible. For example, a more recent study using diabetic rats with characteristic cardiac abnormalities showed a diminished length dependence of Ca^{2+} sensitivity along with a shift in troponin T isoform expression (3). Alternatively, McDonald and Moss (27) have proposed that lateral compression of the contractile proteins resulting from stretch is responsible for the increased Ca^{2+} sensitivity at long sarcomere lengths. They observed that osmotic compression of myocytes at short length could mimic the effects of stretching the myocytes to a longer sarcomere length. This suggested that the tension properties at long sarcomere length were due to compression of the myofilament lattice, although more recent results using direct measurements of lattice spacing (21) indicate that changes in Ca^{2+} sensitivity with osmotic compression cannot be accounted for by changes in lattice spacing. In any case, the results of McDonald and Moss suggest that there may be a mechanical component to the increased Ca^{2+} sensitivity at longer lengths. It is possible that exercise training could induce a change in the structural relationship between thick and thin filaments and result in enhanced cross-bridge interaction. Previous work in skeletal muscle has suggested that contractile protein spacing is subject to alteration as a result of altered muscle activity (42). Further work is necessary to determine the mechanisms of the training induced increase in the length dependence of Ca^{2+} sensitivity.

In conclusion, we have shown for the first time that the length dependence of both maximal tension and the Ca^{2+} sensitivity of submaximal tension is increased at the cellular level in the myocardium as a result of exercise training. This effect may underlie the training-induced enhancement of contractile function in the myocardium and may suggest that this enhancement is more substantial at higher cardiac volumes or longer muscle lengths. Our result suggests that this adaptation occurs at the level of the contractile element, but the mechanism for this adaptation remains unresolved.

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