ARTICLE

Adaptation of Cardiac Myocyte Contractile Properties to Exercise Training

Gary M. Diffee

Biodynamics Laboratory, University of Wisconsin, Madison, WI

DIFFEE, G. M. Adaptation of cardiac myocyte contractile properties to exercise training. *Exerc. Sport Sci. Rev.*, Vol. 32, No. 3, pp. 112–119, 2004. *Recent work suggests that chronic exercise induces alterations in the contractile properties of cardiac myocytes. These alterations include increased sensitivity to activation by Ca^{2+}, changes in the force- length relationship, and increased power output. A recently observed shift in expression of myosin light chain 1 subunit isoforms induced by training may provide a molecular mechanism for these contractile alterations.* **Keywords:** myocyte, Ca^{2+} sensitivity, power output, microarray, atrial myosin light chain

INTRODUCTION

Chronic endurance exercise training has been shown to elicit positive adaptations in the cardiovascular system that result in improved performance at both maximal and submaximal work levels. Among these adaptations in cardiac function is an increase in both submaximal and maximal stroke volume. This training-induced increase in stroke volume may be the result of a number of factors, including increased end-diastolic volume resulting from increased filling time that is the result of training-induced bradycardia, decreased afterload, and enhanced contractile function of the myocardium. Exercise training also has been found to increase peak left-ventricular pressure development in a number of studies. Because pressure development in the ventricle is driven by the capacity of the myocardium to generate tension or force, several studies have examined the effect of exercise training on tension production in ventricular muscle preparations. Exercise training has been shown to result in an increase in isometric tension production in myocardial tissue (7,14), providing evidence that training induces an enhancement of intrinsic myocardial contractile function. Although it is now widely accepted that exercise training results in increased contractility in the myocardium, the identification of specific cellular mechanisms involved in the improved function has proved difficult.

One hypothesis that has been investigated is that this increase in peak tension is brought about by an increase in the level of intracellular Ca^{2+} in the myocardium during activation. Previous work has suggested training-induced alterations in the Ca^{2+} handling processes of the cells (reviewed in (9)). Evidence has been presented suggesting that exercise training alters sarcolemmal Ca^{2+} influx, 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, although they have not found in others. Changes in Ca^{2+} binding and transport by the sarcoplasmic reticulum have been reported in some training studies, but have not been observed in others. However, in addition to these conflicting reports regarding particular Ca^{2+} entry pathways, experiments using single, electrically stimulated myocytes have failed to demonstrate an increase in the height of the intracellular Ca^{2+} transient during activation in cells from trained hearts compared with those from sedentary hearts (6). In fact, the data suggest a decrease in the height of the Ca^{2+} transient (8,15). Thus, the ultimate effect of training on Ca^{2+} levels inside the cell is not clear.

This review focuses on our recent work addressing cardiac adaptation to exercise using permeablized (or "skinned") cardiac myocytes (Fig. 1). This preparation has the advantages of other single-cell preparations, however, in skinned cell preparations, the focus is restricted to investigation into changes at the level of the cardiac contractile element, which can be defined as the array of myofilaments comprised of actin, myosin, and numerous regulatory proteins. Thus, changes in Ca^{2+} handling properties of the sarcolemma and

Address for correspondence: Gary M. Diffee, Biodynamics Laboratory, Department of Kinesiology, 2000 Observatory Drive, University of Wisconsin, Madison, WI 53706 (E-mail: diffee@education.wisc.edu).

Accepted for publication: April 12, 2004.

0091-6331/3203/112–119
Exercise and Sport Sciences Reviews

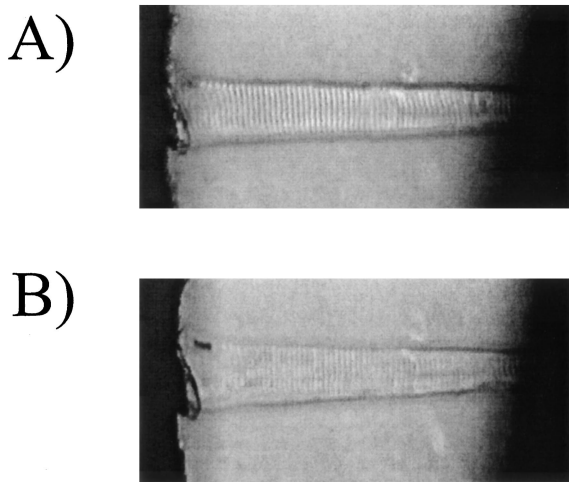

Figure 1. Photomicrograph of a permeablized skinned myocyte mounted between a force transducer and motor positioner. The myocyte is bathed in relaxing solution (pCa 9.0) in (A), and the same myocyte is in maximally activating solution (pCa 4.5) in (B). This cell length was 125 μm between the points of attachment. (Reprinted from Diffee, G.M., E.A. Seversen, and M.M. Titus. Exercise training increases the Ca^{2+} sensitivity of tension in rat cardiac myocytes. *J. Appl. Physiol.* 91:309–315, 2001. Copyright © 2001 The American Physiological Society. Used with permission.)

sarcoplasmic reticulum are eliminated as potential sources of variability, and only those regulatory mechanisms associated with the myofilaments are retained. Although there are certainly a number of regulatory processes that can not be assayed using skinned myocytes, there are a number of important properties of myocardial contraction that do seem to be regulated at the crossbridge or myofilament level. However, except for some earlier studies of training-induced changes in myosin ATPase activity, the effect of exercise training on contractile properties regulated by the contractile element only recently has begun to be investigated. Using a rat treadmill training protocol that previously was used to induce cardiac adaptations in the rat, we examined a number of different contractile properties in skinned cardiac myocytes to explore underlying mechanisms of training-induced improvements in myocardial contractile function.

EFFECT OF EXERCISE TRAINING ON Ca^{2+} SENSTIVITY OF TENSION

Our initial hypothesis was that exercise training may alter the Ca^{2+} sensitivity of myocyte force development. 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 training-induced increase in isometric tension in myocardial tissue. We (1) showed that training increased the sensitivity of myocytes to activation by Ca^{2+} force during submaximal activations. Figure 2 shows the relationship between tension and Ca^{2+} (expressed as pCa, the negative log of the Ca^{2+}) in trained myocytes compared with sedentary myocytes. Although there was no effect of training on maximal tension in these myocytes, substantially more tension was generated at submaximal Ca^{2+} in trained myocytes compared with sedentary myocytes. This resulted in a leftward shift in the tension-pCa relationship in the trained myocytes relative to sedentary. The pCa_{50}, which refers to the Ca^{2+} producing half-maximal tension, is a measure of the Ca^{2+} sensitivity of tension. The pCa_{50} for sedentary myocytes was 5.83, whereas that of trained myocytes was 5.89. In Figure 2B, this data is replotted in bar graph form to illustrate the physiological significance of this increase in Ca^{2+} sensitivity. At a pCa of 6.1, tension was increased from 3% (sedentary) of maximal to 11% (trained) of maximal tension pCa 6.1. At a pCa of 6.0,

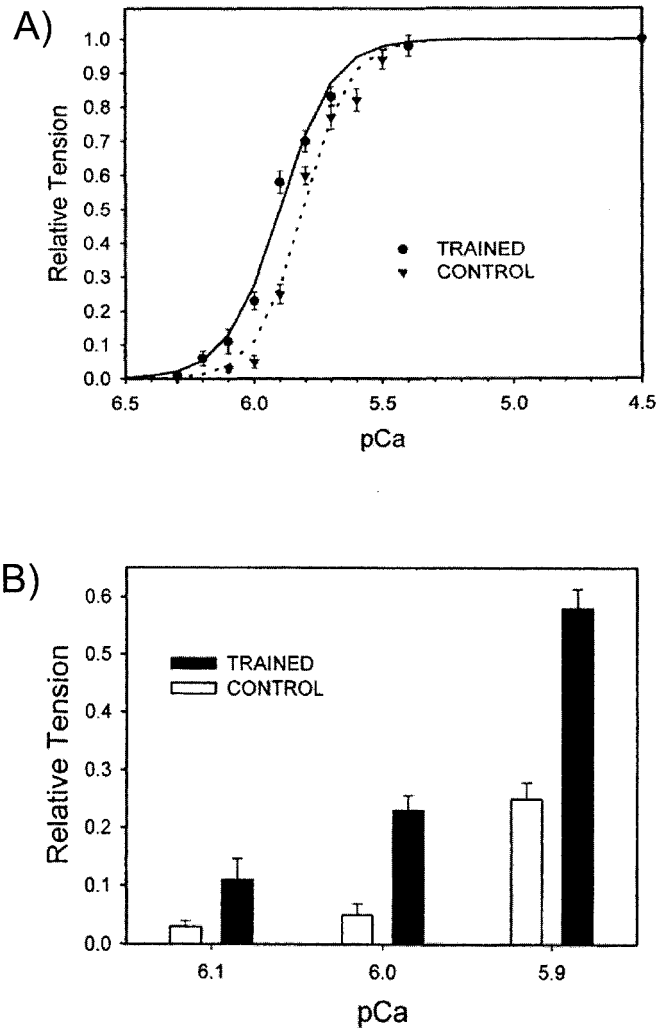

Figure 2. A. Relationship between relative tension and pCa in skinned myocytes. Data were compiled from $N = 70$ control myocytes and $N = 70$ trained myocytes. Relative tension data at each pCa was averaged from all myocytes in the group and are presented as mean ± SD. The trained myocytes exhibited a leftward shift in the tension–pCa relationship compared with sedentary myocytes. The pCa_{50} of these composite curves was 5.83 for control and 5.89 for trained. Sarcomere length was set to an average of 2.35 μm in these cells. B. Data replotted from (A) to highlight the difference in relative tension between trained and control myocytes at three submaximal $[Ca^{2+}]$. Data are presented as mean ± SD. This data illustrate the substantial increase in tension output at low $[Ca^{2+}]$ that is associated with training. (Reprinted from Diffee, G.M., E.A. Seversen, and M.M. Titus. Exercise training increases the Ca^{2+} sensitivity of tension in rat cardiac myocytes. *J. Appl. Physiol.* 91:309–315, 2001. Copyright © 2001 The American Physiological Society. Used with permission.)

tension increased from 5% (sedentary) to 23% (trained) of maximal, and a pCa 5.9 tension increased from 25% (sedentary) to 58% (trained) of maximal. Because much of the cardiac twitch takes place at submaximal Ca^{2+} levels, this increase in tension output during submaximal activations has significant effects on force output during a normal cardiac cycle. This is an important result because it provides a cellular mechanism for increased isometric force associated with exercise training, regardless of the effect of training on Ca^{2+}-handling processes and intracellular Ca^{2+} levels. This result also demonstrates, for the first time, training-induced adaptations in properties of force output regulated at the level of the contractile element. Two recent studies from other laboratories also addressed the effect of exercise training on the Ca^{2+} sensitivity of tension in single cardiac cells using different experimental techniques. Wisloff *et al.* (15) found evidence for an increase in Ca^{2+} sensitivity of tension with exercise training, whereas Natali *et al.* (12) found no evidence for altered Ca^{2+} sensitivity. Possible reasons for this difference are discussed below.

EFFECT OF TRAINING ON LENGTH DEPENDANCE OF TENSION

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. 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. The effect of exercise training on this length dependence has not been addressed at the cellular level. We extended our examination of the effect of exercise training on tension properties in skinned myocytes and showed that training increased both the length dependence of the Ca^{2+} sensitivity and of the length dependence of maximal force (3). We performed Ca^{2+} sensitivity measurements and maximal tension measurements in each cell at two different sarcomere lengths (1.9 and 2.3 μm) that are thought to represent the working range of sarcomere lengths in the intact myocardium. We found that in myocytes from trained hearts, the change in sarcomere length had a much greater effect on both maximal tension and on the Ca^{2+} sensitivity of tension. Maximal tension was not significantly different between trained and sedentary at either sarcomere length. However, exercise training increased the length dependence of maximal tension, because the difference in maximal tension at the two sarcomere lengths was significantly greater in trained myocytes compared with sedentary myocytes. With regard to submaximal tension, the shift in pCa_{50} (increased sensitivity) associated with an increase in sarcomere length was much greater in trained myocytes compared with sedentary myocytes (Fig. 3). The change in length dependence of Ca^{2+} sensitivity was such that at the longer sarcomere length, there was a significant increase in Ca^{2+} sensitivity in trained compared with sedentary myocytes (consistent with our earlier finding), but there was no difference in Ca^{2+} sensitivity between trained and sedentary at the shorter sarcomere length.

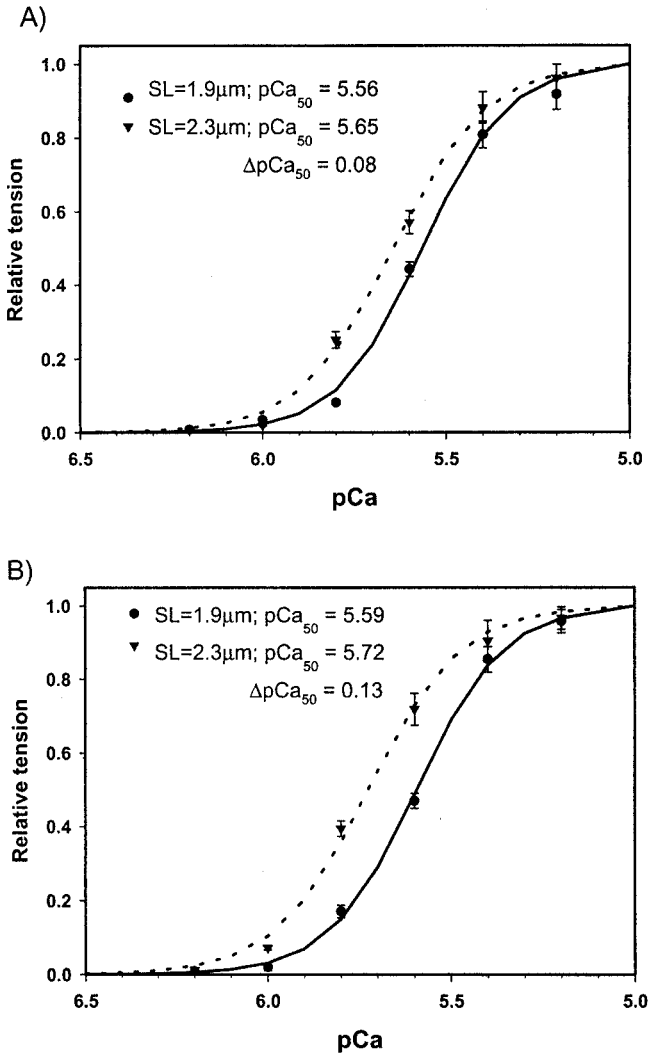

Figure 3. Effect of change in sarcomere length on tension–pCa curves for sedentary (A) and trained (B) myocytes. Data were compiled from 50 sedentary and 50 trained myocytes. Relative tension data at each pCa were averaged at each sarcomere length from all myocytes in each group. Measurements were made at both sarcomere lengths in each myocyte. Data points are presented as mean ± SD. Lines are the best fit regression line using the Hill equation as described in (3). The pCa_{50} values are given for each sarcomere length for the two groups. (Reprinted from Diffee, G.M., and D.F. Nagle. Altered sarcomere length dependence of tension in exercise-trained cardiac myocytes. *J. Appl. Physiol.* 94:1137–1144, 2003. Copyright © 2003 The American Physiological Society. Used with permission.)

The training-induced increase in length dependence of tension has two important implications. First, it provides a cellular mechanism for earlier observations that training increases left ventricular function primarily at high end-diastolic volumes (corresponding to long cell or sarcomere lengths. Second, it provides an explanation of conflicting results regarding effects of training on Ca^{2+} sensitivity of tension. As mentioned above, Natali *et al.* (12) found no effect of training on Ca^{2+} sensitivity. However, these results were obtained with myocytes at a short sarcomere length. We

also found the effect of training to increase Ca^{2+} sensitivity was evident only when the myocytes were at the long sarcomere length (2.3 μm).

EFFECT OF TRAINING ON LOADED SHORTENING AND POWER OUTPUT

A number of studies using intact hearts or isolated working heart preparations have used training-induced increases in stroke volume, increases in peak pressure, or increases in the rate of pressure development as indices of increased myocardial contractile function. However, these various functional parameters all may involve different regulatory mechanisms at the cellular level. Similarly, studies using isolated myocardial preparations or isolated single cells often have used training-induced increases in isometric tension or training-induced increases in unloaded shortening as indices of altered contractile function. The implication is that these changes in contractile properties result in an increase in the pumping capacity of the ventricle. However, much of the cardiac cycle involves the ventricle shortening against a load. At these two extremes of contractile behavior (isometric tension or unloaded shortening), power output (and thus work capacity) is zero. Further, the cellular and molecular properties that regulate unloaded shortening, isometric tension development, and loaded shortening are different and may adapt differently to exercise training. Isometric tension ultimately is limited by the number of active crossbridges, and so is affected by myocyte cross-sectional area or extent of activation. Unloaded shortening velocity, however, is governed by the rate of ATP hydrolysis (specifically limited by the rate of ADP release), which in turn governs the rate of crossbridge cycling. Loaded shortening, and hence power output, is governed by some combination of these two parameters (*i.e.*, number of crossbridges and rate of crossbridge cycling).

To address the lack of information regarding the effect of exercise training on properties directly related to work output of the myocardium, we determined the effect of exercise on force-velocity and power output properties in single myocytes (2). We demonstrated that, although training had no effect on either maximal isometric force or maximal unloaded shortening, training did increase shortening velocity during loaded contractions (Fig. 4A). Because power output is the product of force and shortening velocity, the force velocity curve can be transformed into a force-power curve (Fig. 4B).

The importance of the training-induced increase in loaded shortening velocity is evident in this force-power curve. Peak power output was increased significantly (60% greater) in trained myocytes compared with sedentary myocytes. Power output was increased despite the fact that maximal tension was not significantly increased in trained cells compared with sedentary cells. This is the first direct study of the effect of training on force-velocity properties and power output in single myocytes, and these results provide evidence that exercise training increases the capacity of the myocardium to perform external work. Because it is this capacity to perform external work that likely determines the ability to pump blood against a load, this result suggests that exercise training would increase the ejection of blood against a given afterload.

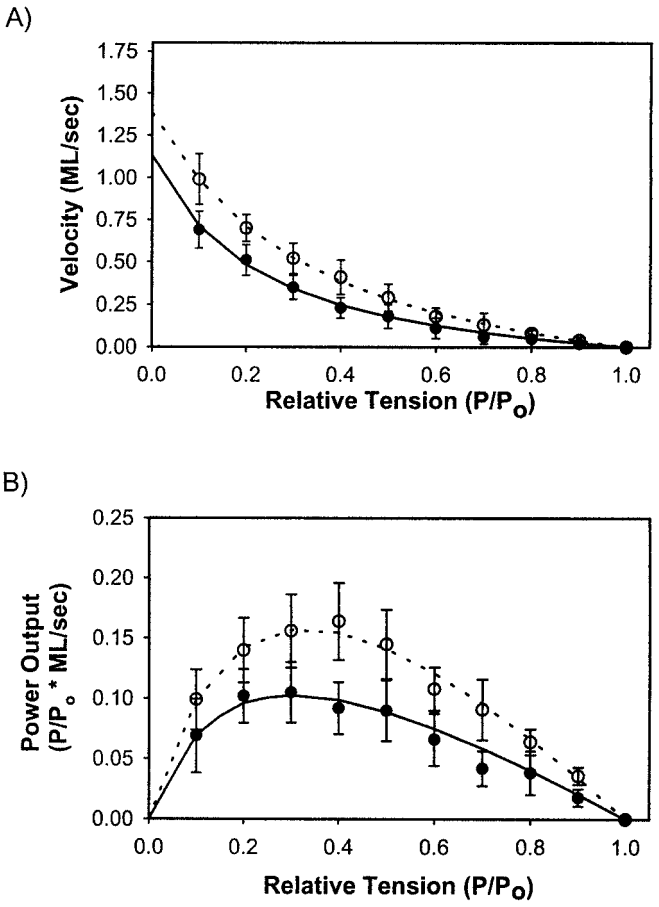

Figure 4. A. Composite force-velocity curves for sedentary and trained myocytes. Data were compiled from 43 sedentary and 46 trained myocytes. Isotonic shortening velocity data at each load were averaged from all myocytes in each group (trained vs. sedentary). Data points are presented as mean ± SD. ○, trained; ●, sedentary. B. Force-power curve constructed from force-velocity data. In each myocyte at each load, force values (expressed as P/P_o) were multiplied times mean velocity values (expressed as ML/s) to result in a value of power output for that load. Data points are mean ± SD for all trained cells and all sedentary cells. ○, trained; ●, sedentary. Peak power output was taken from the highest point in the best fit line. (Reprinted from Diffee, G.M., and E. Chung. Altered single cell force-velocity and power properties in exercise-trained rat myocardium. *J. Appl. Physiol.* 94:1941–1948, 2003. Copyright © 2003 The American Physiological Society. Used with permission.)

MOLECULAR MECHANISMS FOR ALTERED CONTRACTILE FUNCTION

The training-induced changes in contractile properties of skinned myocytes described above suggest the presence of some molecular adaptations underlying these changes in cellular function. Alterations in contractile protein isoform content represent one potential mechanism that may confer altered contractile properties to the myocardium. Because myosin ATPase activity (one of the primary determinants of shortening velocity) varies among myosin heavy chain (MHC) isoforms, changes in MHC isoform expression associated with exercise training have been well studied (reviewed in (9)). There are two MHC isoforms expressed in the vertebrate myocardium, α-MHC and β-MHC. In the adult rat, the α-MHC isoform is thought to predominate, but the

relative distribution of these two isoforms changes in response to development, hormone levels, and disease. Studies examining the effect of endurance exercise training on MHC expression in the heart have yielded conflicting results. A number of studies using swimming as a training method have suggested that exercise training induces an increase in α-MHC expression in the rat heart, although an increase in α-MHC expression also has been observed in rats trained by running. A number of studies using treadmill training have found no evidence for a change in MHC expression in response to exercise training, and a training-induced increase in β-MHC expression has been described. In our lab, we have found no change in MHC isoform content in trained myocardium, despite our finding of increased loaded shortening velocity (3). These conflicting results suggest that it is possible to have significant alterations in myocardial contractile function with no change in MHC isoform expression.

To address other possible molecular mechanisms for observed changes in contractile properties, we took advantage of recently developed microarray expression analysis techniques to assay the effects of training on the expression of more than 8800 genes in the heart (5). We found a number of differences in gene expression changes between trained myocardium and sedentary. These differences are listed in Table 1. The most significant change, from the standpoint of contractile function, was the observed increase in atrial myosin light chain 1 (aMLC1) expression in the ventricles of trained rats. To confirm the microarray results, we performed reverse-transcriptase polymerase chain reaction using a primer specific for aMLC1 and also tested for the presence of aMLC1 protein using two-dimensional gel electrophoresis (Fig. 5). Both of these methods confirmed that aMLC1 expression is increased in ventricular tissue at both the mRNA and protein level as a result of the endurance exercise-training program.

There are two subfamilies of light chains associated with myosin, the essential light chains (ELC; also referred to as MLC1 or MLC3) and the regulatory light chains (also referred to as MLC2). There are multiple isoforms of both ELC and regulatory light chains expressed in rat striated muscle. The expression of these MLC isoforms changes throughout development. The fetal rat heart expresses an embryonic form of the ELC (ELC1emb), which is identical to the atrial MLC1 isoform (aMLC1). In the course of development, light chain expression changes in ventricular tissue to the ventricular isoform (vMLC1), which is identical to the slow skeletal isoform of MLC1. Thus, in the adult rat heart, atrial tissue expresses exclusively aMLC1, whereas ventricular tissue expresses vMLC1.

This pattern of MLC1 expression in ventricular tissue has been shown to change under pathologic conditions. In both human hypertrophic cardiomyopathy (10) and in a porcine model of hypertension (11), aMLC1 expression has been shown to be increased in ventricular myocardium, and this increased expression has been shown to alter contractile properties of the myocardium. In these pathological conditions, the increase in aMLC1 was associated with increased Ca^{2+} sensitivity of tension (10) and increased maximal shortening velocity (11). In addition, transgenic overexpression of aMLC1 in ventricular tissue has been shown to increase both maximum shortening velocity and maximum power output (13).

Our finding of increased aMLC1 expression with exercise training and the remarkable similarity between the functional effects of increased aMLC1 expression under pathological conditions and the altered function associated with exercise training that we have described suggests that this increase in aMLC1 expression may serve as a molecular mechanism for training-induced changes in myocyte contractile function. The mechanism(s) for the effects of increased

TABLE 1

Results of microarray expression analysis of effects of exercise training on cardiac gene expression

Decreases			Increases		
Gene Description	FC	CV	Gene Description	FC	CV
Uncoupling protein 2	−3.1 ± 0.25	0.08	IGF binding protein 3	4.2 ± 0.34	0.09
Monoamine oxidase A	−2.5 ± 1.05	0.42	VEGF receptor 2/FLK-1	2.4 ± 0.95	0.40
Decorin	−1.7 ± 0.82	0.48	Atrial myosin light chain 1	2.7 ± 1.12	0.41
Eukaryotic translation factor 1 α 2	−3.2 ± 1.75	0.55	β globin gene	1.7 ± 0.73	0.43
Na/K-ATPase α-1 subunit	−1.8 ± 0.33	0.18	Laminin chain β 2	3.0 ± 1.05	0.35
Skeletal muscle alpha actin	−2.2 ± 1.21	0.55	β-actin	2.2 ± 1.26	0.57
Heat shock protein 70	−2.8 ± 1.10	0.39	Glutathione-S-transferase	1.6 ± 0.67	0.42
Pyruvate dehydrogenase phosphatase enzyme	−4.3 ± 1.33	0.21	Cytochrome P450	2.5 ± 0.73	0.29
GLUT1 glucose transporter	−2.0 ± 1.07	0.54	Rat pancreatitis associated protein	2.3 ± 1.36	0.59
Gamma atrial natriuretic peptide precursor	−1.9 ± 0.48	0.25	2,4-dienoyl-CoA reductase	1.5 ± 0.42	0.28
Cardionatrin precursor	−2.3 ± 0.53	0.13			
Atrial natriuretic factor	−2.2 ± 1.4	0.64			
Glutamine synthetase	−3.3 ± 0.87	0.26			
Calreticulin	−2.7 ± 1.16	0.43			
Phosphatidylinositol transfer protein	−1.5 ± 0.94	0.63			
Dihydrolipoamide acetyltransferase	−1.6 ± 0.46	0.29			
Plasma glutathione peroxidase	−2.1 ± 1.22	0.58			

Genes that were significantly decreased or increased in trained compared with sedentary samples. FC, average fold change in expression (mean ± SD); CV, coefficient of variation (SD/mean).

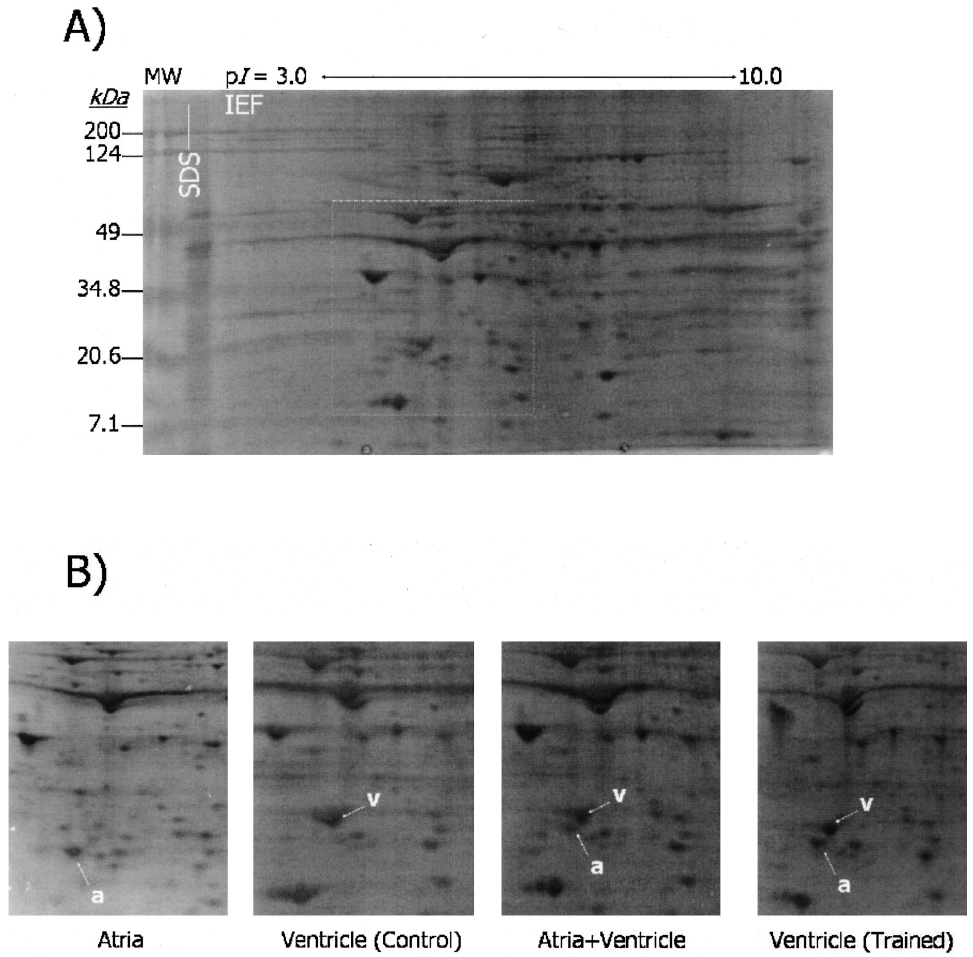

Figure 5. Results of two-dimensional electrophoresis analysis of aMLC1 protein expression in trained and sedentary ventricular tissue. A. Whole-gel results of first dimension isoelectric focusing (IEF) using a pH 3–10 gradient and second dimension SDS-PAGE with 12.5% acrylamide gel. B. Close-up of aMLC1/vMLC1 region of gels (box in (A)) used for analysis. Shown are representative gels using homogenates from sedentary atrial tissue, control ventricular tissue, a mixture of atrial and ventricular homogenates, and trained ventricular tissue. a, aMLC1; v, vMLC1. Identification of aMLC1 and vMLC1 was based on predicted isolectric point and molecular weight values for these proteins as well as previously published two-dimensional electrophoretic analyses of these proteins. (Reprinted from Diffee, G.M., E.A. Seversen, T.D. Stein, and J.A. Johnson. Microarray expression analysis of effects of exercise training: increase in atrial MLC-1 in rat ventricles. *Am. J. Physiol.* 284:H830–H837, 2003. Copyright © 2003 The American Physiological Society. Used with permission.)

aMLC1 expression to alter myocardial contractility was not known. It has been demonstrated that the N-terminal region of the MLC1 molecule interacts with the actin filament during crossbridge formation. Sequence differences between aMLC1 and vMLC1 result in a difference in charge in this N-terminal region that may affect the ability of the light chain to bind to actin (10).

PHYSIOLOGICAL VERSUS PATHOLOGICAL HYPERTROPHY

The similarity of these different stressors (pathological overload vs exercise training) to induce similar changes in the expression of aMLC1 suggests the possibility of common pathways of adaptation. One thing both of these stressors have in common is a degree of cardiac hypertrophy. A treadmill training program typically elicits moderate cardiac hypertrophy, as evidenced by an increase in the heart weight-to-body weight ratio of 10–20%. More severe hypertrophy is a hallmark response to pathological conditions such as pressure overload and cardiomyopathies. Previous reports have indicated that, under these pathological hypertrophic stimuli, a so-called *fetal gene program* is induced that includes the downregulation of adult isoforms of several cardiac proteins and the upregulation of genes such as β MHC, skeletal α actin, atrial natriuretic factor, Glut1 glucose transporter, and aMLC1. In our microarray results, we found no evidence for the induction of any of these fetal genes, with the exception of aMLC1. In fact, we found a decrease in expression of several of these genes previously related to hypertrophy, including atrial natriuretic factor, skeletal α actin, and Glut1 glucose transporter. These results emphasize the complex relationship between cardiac hypertrophy induced by a number of different stressors.

Hypertrophic growth of the myocardium in response to stressors such as pressure overload was thought initially to be an adaptive response that temporarily augments or maintains cardiac output in the face of increased load. However, this growth eventually results in decreased

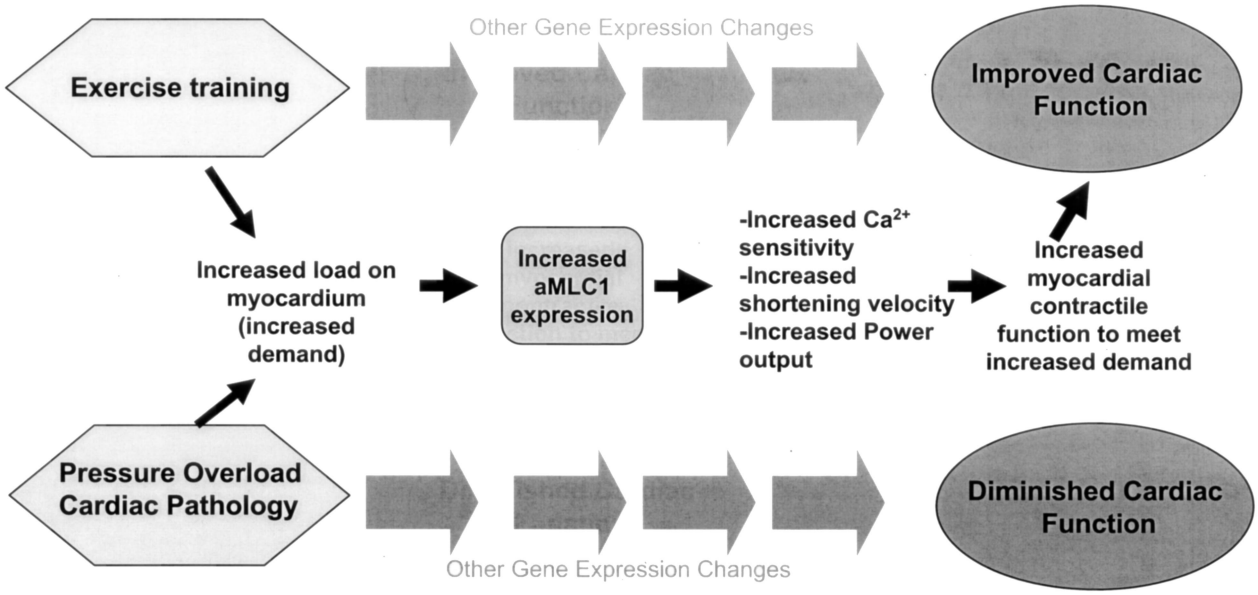

Figure 6. Schematic suggesting a relationship between adaptations to exercise training versus adaptations to pathological conditions. Both stressors are suggested to result in increased load and thus an increase in demand placed on the myocardium. One response to this increased demand is an increase in aMLC1 expression that enhances myocardial contractile properties to meet the increased demand. This and other changes in gene expression result in improved cardiac function in the case of exercise training. In the case of pathological stressors, the improvement in function allows the myocardium initially to meet the demand, but the chronic nature of the increased demand as well as other changes in gene expression ultimately results in a decrease in cardiac function.

cardiac function. The similarity of the change in MLC1 expression between pathological conditions and exercise training suggests that there may be some common pathways associated with these two hypertrophic responses. However, it seems clear that there must be different pathways ultimately involved, as evidenced by the very different gene expression profiles and by the fact that improved cardiac function as a result of exercise training is usually maintained. Figure 6 summarizes possible similarities and differences in different hypertrophic responses.

REGIONAL DIFFERENCES IN TRAINING ADAPTATIONS

The identification of a molecular marker for improved contractile function associated with training provided the opportunity to determine regional variations within the wall of the myocardium in the effects of exercise training. It is clear that there are a number of regional differences in myocardial properties. Electrical properties are known to vary across different regions of the ventricle and even across the width of the ventricular wall within the same region. Mechanical and biochemical properties also have been shown to be different in the subendocardial (ENDO) region of the myocardium compared with the subepicardial region (EPI). Several studies recently pointed out that the ENDO region of the myocardium is more responsive to adaptation to a number of stimuli, including exercise training. We recently showed that the training-induced increase in Ca^{2+} sensitivity of tension was more pronounced in cells from the ENDO region (4). In addition, we described a greater training-induced increase in aMLC1 expression in ENDO tissue compared with EPI tissue. This result is important because it may provide clues regarding the mechanism of adaptation, because many of the differences between ENDO myocardium and EPI myocardium are thought to be the result of differences in pressure load on these different regions. In addition, we found that there was a significant correlation between variation in Ca^{2+} sensitivity and variation in aMLC1

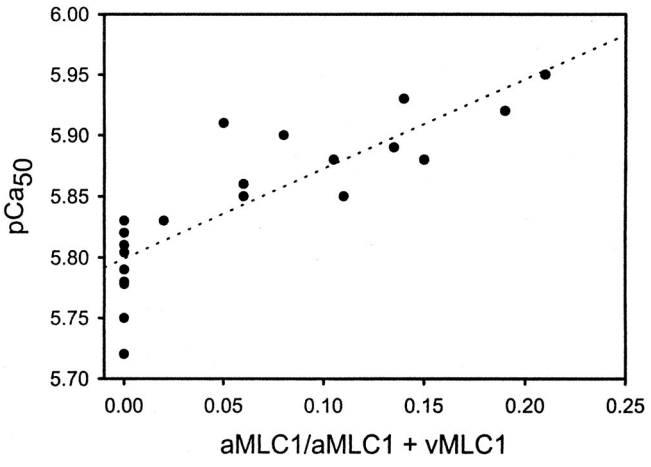

Figure 7. Correlation between pCa_{50} of cardiac myocytes and the aMLC1 content of the tissue from which the myocytes were isolated (ENDO vs EPI). aMLC1 content is expressed as a fraction of total MLC1 (aMLC1 + vMLC1). vMLC1, ventricular isoform of MLC1. pCa_{50} values are means from a minimum of five cells per group. The slope of the regression line is 0.74. The correlation coefficient (r^2) is 0.72, which represents a significant correlation ($P < 0.01$). (Reprinted from Diffee, G. M., and D. F. Nagle. Regional differences in effects of exercise training on contractile and biochemical properties of rat cardiac myocytes. *J. Appl. Physiol.* 95: 35–42, 2003. Copyright © 2003 The American Physiological Society. Used with permission.)

expression, lending further support to the role of increased aMLC1 expression as a molecular mechanism for training-induced improvements in myocardial contractile function (Fig. 7).

CONCLUSIONS

Taken together, these findings provide evidence for significant adaptation to exercise training at the level of the contractile element. These adaptations provide a cellular mechanism for the improved contractile function of the ventricular myocardium that accompanies exercise training. The increase in aMLC1 expression may provide a molecular mechanism that increases submaximal tension and power output in the myocardium. In addition, these cellular and molecular adaptations may provide clues regarding both common and distinct signaling pathways that govern the response to exercise training compared with the response to other hypertrophic stimuli. There is the need for more work regarding the additional alterations in single-cell contractile properties. There is also the need for more work determining other molecular mechanisms underlying functional changes. In addition to changes in contractile protein isoform expression, phosphorylation of contractile proteins is a key mechanism by which mechanical properties are regulated in the heart. Among others, troponin I, C-protein, and the regulatory light chain of myosin are phosphorylated by a specific kinases. Phosphorylation of these proteins alters tension and shortening properties in cardiac myocytes and thus represents a potential molecular mechanism underlying functional adaptations to exercise training. However, little is known regarding the effects of training on these protein modifications. Study of the cellular and molecular adaptations to exercise training is important in furthering our understanding of the role of exercise training in ameliorating the compromised myocardial function that is associated with aging and cardiac pathologies.(6,8)

References

1. Diffee, G.M., E.A. Seversen, and M.M. Titus. Exercise training increases the Ca^{2+} sensitivity of tension in rat cardiac myocytes. *J. Appl. Physiol.* 91:309–315, 2001.
2. Diffee, G.M., and E. Chung. Altered single cell force-velocity and power properties in exercise-trained rat myocardium. *J. Appl. Physiol.* 94:1941–1948, 2003.
3. Diffee, G.M., and D.F. Nagle. Exercise training alters length dependence of contractile properties in rat myocardium. *J. Appl. Physiol.* 94:1137–1144, 2003.
4. Diffee, G.M., and D.F. Nagle. Regional differences in effects of exercise training on contractile and biochemical properties of rat cardiac myocytes. *J. Appl. Physiol.* 95:35–42, 2003.
5. Diffee, G.M., E.A. Seversen, T.D. Stein, and J.A. Johnson. Microarray expression analysis of effects of exercise training: increase in atrial MLC-1 in rat ventricles. *Am. J. Physiol.* 284:H830–H837, 2003.
6. Laughlin, M.H., M.E. Schaefer, and M. Sturek. Effect of exercise training and intracellular free Ca^{2+} transients in ventricular myocytes of rats. *J. Appl. Physiol.* 73:1441–1448, 1992.
7. Molè, P. Increased contractile potential of papillary muscles from exercise trained rat hearts. *Am. J. Physiol.* 234:421–425, 1978.
8. Moore, R.L., T.I. Musch, R.V. Yelamarty, R.C. Scaduto Jr., A.M. Semanchick, M. Elensky, and J.Y. Cheung. Chronic exercise alters contractility and morphology of isolated rat cardiac myocytes. *Am. J. Physiol.* 264:C1180–C1189, 1993.
9. Moore, R.L., and D.H. Korzick. Cellular adaptations of the myocardium to chronic exercise. *Prog. Cardiovasc. Diseases* 37:371–396, 1995.
10. Morano, I., K. Hadicke, H. Haase, M. Bohm, E. Erdmann, and M.C. Schaub. Changes in essential myosin light chain isoform expression provide a molecular basis for isometric force regulation in the failing human heart. *J. Mol. Cell. Cardiol.* 29:1177–1187, 1997.
11. Morano, M., P. Boels, S.G. Haworth, H. Haase, and I. Morano. Expression and function of atrial myosin light chain1 in the porcine right ventricle of normal and pulmonary hypertensive animals. In: *Mechanism of Work Production and Work Absorption in Muscle*, edited by H. Sugi and G.H. Pollack. New York: Plenum Press, 1998.
12. Natali, A.J., L.A. Wilson, M. Peckham, D.L. Turner, S.M. Harrison, and E. White. Different regional effects of voluntary exercise on the mechanical and electrical properties of rat ventricular myocytes. *J. Physiol.* 541:863–875, 2002.
13. Sanbe, A., J. Gulick, E. Hayes, D. Warshaw, H. Osinska, C.B. Chan, R. Klevitsky, and J. Robbins. Myosin light chain replacement in the heart. *Am. J. Physiol.* 279:H1355–H1364, 2000.
14. Tibbits, G.F., R.J. Barnard, K.M. Baldwin, N. Cugalj, and N.K. Roberts. Influence of exercise on excitation-contraction coupling in rat myocardium. *Am. J. Physiol.* 240:H472–H480, 1981.
15. Wisloff, U., J.P. Loennechen, G. Falck, V. Beisvag, S. Currie, G. Smith, and O. Ellingsen. Increased contractility and calcium sensitivity in cardiac myocytes from endurance trained rats. *Cardiovasc. Res.* 50:495–508, 2001.