Microarray expression analysis of effects of exercise training: increase in atrial MLC-1 in rat ventricles

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Diffee, Gary M., Eric A. Seversen, Thor D. Stein, and Jeffrey A. Johnson. Microarray expression analysis of effects of exercise training: increase in atrial MLC-1 in rat ventricles. Am J Physiol Heart Circ Physiol 284: H830–H837, 2003. First published November 7, 2002; 10.1152/ajpheart.00761.2002.—Previous studies have shown that endurance exercise training increases myocardial contractility. We have previously described training-induced alterations in myocardial contractile function at the cellular level, including an increase in the Ca2+ sensitivity of tension. To determine the molecular mechanism(s) of these changes, oligonucleotide microarrays were used to analyze the gene expression profile in ventricles from endurance-trained rats. We used an 11-wk treadmill training protocol that we have previously shown results in increased contractility in cardiac myocytes. After the training, the hearts were removed and RNA was isolated from the ventricles of nine trained and nine control rats. With the use of an Affymetrix Rat Genome U34A Array, we detected altered expression of 27 genes. Several genes previously found to have increased expression in hypertrophied myocardium, such as atrial natriuretic factor and skeletal α-actin, were decreased with training in this study. From the standpoint of altered contractile performance, the most significant finding was an increase in the expression of atrial myosin light chain 1 (αMLC-1) in the trained ventricular tissue. We confirmed microarray results for αMLC-1 using RT-PCR and also confirmed a training-induced increase in αMLC-1 protein using two-dimensional gel electrophoresis. αMLC-1 content has been previously shown to be increased in human cardiac hypertrophy and has been associated with increased Ca2+ sensitivity of tension and increased power output. These results suggest that increased expression of αMLC-1 in response to training may be responsible, at least in part, for previously observed training-induced enhancement of contractile function.

atrial natriuretic factor; myosin regulatory light chain; calcium sensitivity

CHRONIC EXERCISE TRAINING is known to elicit a number of adaptations in the heart that result in improved cardiac performance. Increased maximal and submaximal stroke volume are thought to be the result, in part, of improvements in myocardial contractile performance (17). Altered force and shortening velocity properties have been demonstrated in myocardial muscle preparations (16, 36), and the contractile properties of single cardiac myocytes have been recently shown to be altered by training (5, 6, 18, 22, 39). We have recently shown that the Ca2+ sensitivity of tension was increased as a result of training in permeabilized cardiac myocytes (6), a result also seen in intact myocytes (39). In addition, the length dependence of tension properties has been shown to be increased by exercise training in permeabilized as well as intact myocytes (5, 22). These results suggest that training induces an adaptation within the myofibrillar contractile apparatus rather than, or in addition to, adaptations that have been hypothesized in other subcellular processes (for a review, see Ref. 17). However, there is no information available regarding training-induced changes within the contractile element that provides a mechanism for our results in single cardiac myocytes.

There are a number of molecular factors that are known to affect contractile properties in the myocardium and that might be altered in the course of an exercise training program. In addition to factors such as phosphorylation of regulatory proteins such as myosin regulatory light chain (RLC) (34) and troponin I (41), altered expression of contractile protein isoforms may provide a mechanism for training-induced enhancement of contractile performance. For example, the Ca2+ sensitivity of tension in cardiac muscle is known to be influenced by the presence of different isoforms of tropomyosin (40), troponin I and T (21, 31), and myosin light chains (MLCs) (19). There is currently no information regarding the effect of exercise training on the expression any of these contractile protein isoforms.

Analysis of gene expression using oligonucleotide microarrays allows for the simultaneous assessment of the expression levels for a large number of genes. Previous work has described the effects of exercise training on the expression of a small number of selected genes in the heart (1, 3, 12, 39), but the results...
of these studies have not clarified possible mechanisms for altered contractile function. In this study, we used microarray expression analysis to compare differences in expression levels for 8,800 genes in control rat ventricular myocardium compared with ventricular tissue from rats trained with an endurance training program. Results of the expression analysis revealed that training increases the expression of atrial MLC1 (aMLC-1) in ventricular myocardium. This change in protein expression could account for a number of previously described training-induced changes in myocardial contractile function.

METHODS

Exercise training protocol. Female Sprague-Dawley rats, 145–175 g initial body wt, were randomly divided into two groups (n = 9 animals/group): sedentary control and exercise trained. All animals were individually housed in a temperature- and light-controlled room (reversed 12:12-h light-dark cycle) with food and water ad libitum. Rats in the exercise-trained group underwent an 11-wk treadmill training protocol that has been previously shown to induce improvements in whole heart function (8), increases in papillary muscle isometric tension (36), and improvements in single myocyte contractile function (6). The rats were trained 5 days/wk with training initially for 15 min/day at 13 m/min and 10% grade. Duration and intensity increased weekly during weeks 1–5 until the animals were running for 1 h at 26 m/min and 20% grade. This workload level was maintained for the remainder of the 11-wk protocol. The University of Wisconsin-Madison Animal Use and Care Committee approved this protocol.

Oligonucleotide microarray experiments. After the completion of the training program, rats were euthanized no less than 72 h after the last exercise bout to avoid any acute effects of exercise. Rats were anesthetized by inhalation of methoxyflurane, and the hearts were quickly removed and placed in ice-cold Ca2+-free relaxing solution. Atria were trimmed away, and the ventricular tissue was blotted dry, weighed, and then divided sagittally into three sections. The sections were quick frozen in liquid nitrogen and stored at −80°C for later RNA isolation. For RNA isolation, a 30-mg piece was cut from each of the three sections for each heart. Frozen sections were homogenized with a Tekmar Tissuemizer in TRIzol reagent (Invitrogen), and total RNA was extracted. An RNaseasy Mini Kit (Qiagen) was used to further purify total RNA. RNA from three hearts from each group of animals was pooled to yield three control group RNA samples and three trained group samples. Double-stranded cDNA was synthesized from the pooled total RNA using a SuperScript choice kit (Invitrogen) with a T7(−dT)15 primer incorporating a T7 RNA polymerase promoter (Integrated DNA Technologies). cDNA was prepared and biont labeled by in vitro transcription (Enzo Biochemical). Labeled RNA was fragmented by incubation at 94°C for 35 min in the presence of 40 mM Tris-OAc (pH 8.1), 100 mM KOAc, and 30 mM MgOAc. Labeled, fragmented cRNA (15 μg) was hybridized for 16 h at 45°C to a U34A rat genome array (Affymetrix). After hybridization, the gene chips were automatically washed and stained with streptavidin-phycocerythrin using a fluidics station. The probe arrays were scanned at 5-μm resolution using a Genechip System confocal scanner made for Affymetrix by Aligent.

Microarray analysis. Affymetrix Microarray Suite software (version 5.0) was used to scan and analyze the relative abundance of each gene based on the intensity of the signal from each probe set. Analysis parameters used by the software were set to values corresponding to moderate stringency (statistical difference threshold = 30, statistical ratio threshold = 1.5). Output from the microarray analysis was merged with the Unigene or GenBank descriptor and saved as an Excel data spreadsheet. We ran three arrays for each group (trained vs. control). The RNA from three animals was pooled for each array, and the comparisons were crossed such that each trained animal set was compared with each control animal set for a total of nine comparisons (3 × 3 matrix). The comparison analysis compares each probe set on one array to its counterpart on another array. For each comparison, the analysis using the Affymetrix software generates a “difference call” of either no change, marginal increase/decrease, or increase/decrease. We then defined “increase, decrease, or no change” of expression for individual genes based on ranking of these difference calls from the 3 × 3 matrix using a method previously described by Li and Johnson (13). Briefly, we set no change = 0, marginal increase = 1, increase = 2, marginal decrease = −1, and decrease = −2. The final rank was determined as the sum of the nine values corresponding to individual probe sets with all values varied from −18 to 18. The cutoff value for increase or decrease was set as ±n2 (n = 3) because of the marginal calls. This means that a gene must be called a marginal increase in all nine comparisons to rank 9 and be on the list. Similarly, a gene called increased in five of nine comparisons (a majority) would rank 10 and be on the list, whereas a gene called increased in four of nine comparisons (a minority) would rank 9 and not be on the list. Therefore, the cutoff in the 3 × 3 matrix would be n2 = 9/−9 (13, 14). The reproducibility of paired comparisons was based on the coefficient of variation (CV; SD/mean) for the fold change (FC) on the ranked genes. A distribution curve of the CV was used to determine a CV cutoff value. The cutoff value was CV < 0.7. Gene categorization was based on the NetAffx database (http://www.NetAffx.com).

RT-PCR. Verification of changed expression was done by RT-PCR for two selected genes. Unique PCR primers specific for the genes of interest were designed using PRIMER3 software (available online at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and using sequence data from the National Center for Biotechnology Information database. Primers (Integrated DNA Technologies) were designed for aMLC-1 (5′-CCA AGC CTG AAG AGA TGA GTA AT′-3′ and 5′-CCA GTA TGA TGC CAG TGC TC′-3′) and atrial natriuretic peptide (ANP; 5′-TTC AAG AAC CTG GTA TTC CT′-3′ and 5′-GCT CCA ATC CTG TCA ATC CT′-3′). Single-stranded cDNA was created by RT (Promega Reverse Transcription System) from total RNA originally prepared for the microarrays. The reaction mix contained 1 μg total RNA, 0.5 μg oligo(dT)15 primer (provided), 1 mM each dNTP, and 15 units avian myeloblastosis virus reverse transcriptase. The RT proceeded for 1 h at 42°C. The PCR reaction mix contained 20% of the RT reaction, 50 pmol gene-specific primers, 200 μM dNTPs, 2 mM MgCl2, and 2.5 units Taq polymerase. DNA was amplified by an initial incubation at 94°C for 1.25 min followed by 21–27 cycles of 94°C for 0.5 min, 54°C for 0.5 min, 72°C for 0.5 min, and a final extension at 72°C for 6 min. The PCR products were separated by electrophoresis in a 1.2% agarose gel and visualized with ethidium bromide staining. Relative intensities were quantified using UN-SCAN-IT software (Silk Scientific).

Two-dimensional gel electrophoresis. To confirm the effect of training on aMLC-1 protein levels, we analyzed homogenates from control and trained heart samples using two-dimensional (2-D) gel electrophoresis. Pieces of frozen tissue from the same hearts from which RNA was isolated were
homogenized at 100 mg/ml in sample buffer (8 M urea, 2 M thiourea, 75 mM DTT, and 10 mM Tris; pH 7.0) using a Fisher PowerGen 700 homogenizer with a 7-mm sawtooth generator at 20,000 rpm for 8–10 s. The protein concentration of this homogenate was determined using a Bio-Rad protein assay kit with BSA as the standard. Isoelectric focusing was performed using Bio-Rad Protein isolectric focusing cell and 11-cm precast immobilized pH gradient (pI) gel. The locations of the α-MLC-1 and ventricular MLC-1 isoforms were visualized using a zinc stain (Pierce) and digitized using a Kodak Image Station 440CF. Sixteen exposures were summed to increase the signal-to-noise ratio. PDQuest software (version 6.0, Bio-Rad) was used for gel image analysis.

The results of the microarray analysis are given in Table 2. The Affymetrix algorithm for detecting differential expression considers several parameters described in the Statistical Algorithms Reference Guide supplement to the Microarray Suite version 5.0 User’s Guide. With the use of the rank analysis described above in METHODS, we originally identified 63 genes or expressed sequence tags whose expression was increased or decreased in trained samples compared with control samples. We eliminated genes with a mean FC coefficient of variation (SD/mean). 3

### Table 1. Effect of exercise training program on body weight, heart weight, and skeletal muscle enzyme activity

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>163.5 ± 1.7</td>
<td>160.5 ± 1.8</td>
</tr>
<tr>
<td>Before training program</td>
<td>163.5 ± 1.7</td>
<td>160.5 ± 1.8</td>
</tr>
<tr>
<td>After training program</td>
<td>275.6 ± 4.3</td>
<td>267.7 ± 2.3</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>736 ± 15</td>
<td>790 ± 14*</td>
</tr>
<tr>
<td>Heart weight/body weight</td>
<td>2.68 ± 0.04</td>
<td>2.95 ± 0.05*</td>
</tr>
<tr>
<td>Plantaris citrate synthase activity, μmol·min⁻¹·g wet wt⁻¹</td>
<td>15.5 ± 1.7</td>
<td>22.6 ± 1.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from the corresponding control value (P < 0.05).

**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 mass 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 46% higher citrate synthase activity compared with control plantaris muscle. The heart mass, heart weight-to-body weight ratio, and plantaris muscle citrate synthase activity were all significantly different than control and are consistent with results of previous studies (5, 6, 18).

The results of the microarray analysis are given in Table 2. The Affymetrix algorithm for detecting differential expression considers several parameters described in the Statistical Algorithms Reference Guide supplement to the Microarray Suite version 5.0 User’s Guide. With the use of the rank analysis described above in METHODS, we originally identified 63 genes or expressed sequence tags whose expression was increased or decreased in trained samples compared with control samples. We eliminated genes with a mean FC

### Table 2. Results of microarray expression analysis

<table>
<thead>
<tr>
<th>Gene Description</th>
<th>R</th>
<th>FC</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decreases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncoupling protein 2</td>
<td>−18</td>
<td>−3.1 ± 0.25</td>
<td>0.08</td>
</tr>
<tr>
<td>Monoamine oxidase A</td>
<td>−18</td>
<td>−2.5 ± 1.05</td>
<td>0.42</td>
</tr>
<tr>
<td>Decorin</td>
<td>−18</td>
<td>−1.7 ± 0.82</td>
<td>0.48</td>
</tr>
<tr>
<td>Eukaryotic translation factor 1c2</td>
<td>−18</td>
<td>−3.2 ± 1.75</td>
<td>0.55</td>
</tr>
<tr>
<td>Na⁺-K⁺-ATPase, α₁-subunit</td>
<td>−14</td>
<td>−1.8 ± 0.33</td>
<td>0.18</td>
</tr>
<tr>
<td>Skeletal muscle α-actin</td>
<td>−14</td>
<td>−2.2 ± 1.21</td>
<td>0.55</td>
</tr>
<tr>
<td>Heat shock protein 70</td>
<td>−14</td>
<td>−2.8 ± 1.10</td>
<td>0.39</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase phosphatase enzyme</td>
<td>−12</td>
<td>−4.3 ± 1.33</td>
<td>0.21</td>
</tr>
<tr>
<td>GLUT1 glucose transporter</td>
<td>−12</td>
<td>−2.0 ± 1.07</td>
<td>0.54</td>
</tr>
<tr>
<td>γ-Atrial natriuretic peptide precursor</td>
<td>−12</td>
<td>−1.9 ± 0.48</td>
<td>0.25</td>
</tr>
<tr>
<td>Cardionatriin precursor</td>
<td>−12</td>
<td>−2.3 ± 0.53</td>
<td>0.13</td>
</tr>
<tr>
<td>Atrial natriuretic factor</td>
<td>−12</td>
<td>−2.2 ± 1.4</td>
<td>0.64</td>
</tr>
<tr>
<td>Glutamine synthetase</td>
<td>−10</td>
<td>−3.3 ± 0.87</td>
<td>0.26</td>
</tr>
<tr>
<td>Calreticulin</td>
<td>−10</td>
<td>−2.7 ± 1.16</td>
<td>0.43</td>
</tr>
<tr>
<td>Phosphotyidylinositol transfer protein</td>
<td>−10</td>
<td>−1.5 ± 0.94</td>
<td>0.63</td>
</tr>
<tr>
<td>Dihydropyridine acetyltransferase</td>
<td>−10</td>
<td>−1.6 ± 0.46</td>
<td>0.29</td>
</tr>
<tr>
<td>Plasma glutathione peroxidase</td>
<td>−10</td>
<td>−2.1 ± 1.22</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Increases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF binding protein 3</td>
<td>18</td>
<td>4.2 ± 0.34</td>
<td>0.09</td>
</tr>
<tr>
<td>VEGF receptor 1/1.1</td>
<td>16</td>
<td>2.4 ± 0.95</td>
<td>0.40</td>
</tr>
<tr>
<td>Atrial myosin light chain 1</td>
<td>16</td>
<td>2.7 ± 1.12</td>
<td>0.41</td>
</tr>
<tr>
<td>β-Globin gene</td>
<td>14</td>
<td>1.7 ± 0.73</td>
<td>0.43</td>
</tr>
<tr>
<td>Laminin chain-β2</td>
<td>14</td>
<td>3.0 ± 1.05</td>
<td>0.35</td>
</tr>
<tr>
<td>β-Actin</td>
<td>12</td>
<td>2.2 ± 1.26</td>
<td>0.57</td>
</tr>
<tr>
<td>Glutathione-S-transferase</td>
<td>12</td>
<td>1.6 ± 0.67</td>
<td>0.42</td>
</tr>
<tr>
<td>Cytochrome P-450</td>
<td>10</td>
<td>2.5 ± 0.73</td>
<td>0.29</td>
</tr>
<tr>
<td>Rat pancreatitis-associated protein</td>
<td>10</td>
<td>2.3 ± 1.36</td>
<td>0.28</td>
</tr>
<tr>
<td>2,4-Dienoyl-CoA reductase</td>
<td>10</td>
<td>1.5 ± 0.42</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Genes that were significantly increased or decreased in trained compared with control samples are shown. R, ranking from 3 × 3 comparison as described in METHODS; FC, average fold change in expression (means ± SD); CV, coefficient of variation (SD/mean).
of <1.5 as well as genes in which the CV for the FC (SD/mean) was >0.7. These cutoff values provide a conservative estimate of the numbers of genes whose expression level is altered by exercise training. The genes that met the final exclusion criteria are listed in Table 2.

**RT-PCR experiments.** To verify the results of the microarray analysis, we examined the expression levels of two selected genes using RT-PCR. We chose the ANP gene because previous studies had yielded conflicting results of the effects of exercise training on the expression of this gene in ventricular tissue (1–3, 12, 15). We chose the aMLC-1 gene because altered expression of this gene provides a potential molecular mechanism for previously observed changes in cellular contractile function. Figure 1 shows the results of agarose gel electrophoresis of the products of the RT-PCR using primers specific for ANP (A) and aMLC-1 (B). The results of the RT-PCR experiments confirmed the microarray analysis results, that is, ANP gene expression is decreased in trained compared with control hearts and aMLC-1 expression is increased in trained compared with control ventricular tissue.

**2-D electrophoresis analysis.** To determine whether the increase in aMLC-1 expression demonstrated by microarray analysis and RT-PCR actually resulted in increased aMLC-1 protein levels in the ventricles of trained animals, we performed 2-D electrophoretic analysis. This method has been used previously to separate the ventricular and atrial isoforms of MLC-1 in human (19, 25) and porcine (20) myocardium. A representative 2-D gel is shown in Fig. 2. The highlighted area was analyzed for the presence of aMLC-1 and vMLC-1 based on the predicted pi and molecular weight of these two isoforms. A magnified image of this region of the gel is shown in Fig. 2B. We performed 2-D gel analysis on atrial samples, a mixture of atrial and ventricular samples, and six trained and six control ventricular samples. Identification of spots corresponding to aMLC-1 and vMLC-1 was based on pi and molecular weight information as well as previously published determinations of aMLC-1 vs. vMLC-1 positions on 2-D gels (20, 25). The predicted pi and molecular mass values of rat aMLC-1 are 4.97 and 21,150.99 Da, respectively, whereas the pi and molecular values of vMLC-1 are 5.03 and 22,025.01 Da (http://us.expasy.org). Densities of spots corresponding to aMLC-1 and vMLC-1 were quantified using PDQuest software, and the amount of each MLC-1 was expressed as a percentage of the total MLC-1 in that sample. Mean data for 3 animals from each group showed that there was no detectable aMLC-1 protein in control ventricles, whereas in trained animals aMLC-1 increased to 13.4 ± 2.1% of the total MLC-1. This difference is statistically significant (P < 0.05).

**DISCUSSION**

Previous studies indicate that endurance exercise training improves contractile function in the myocardium. One possible mechanism for this adaptation would be alterations in the expression of genes related to contractile function, but these possible molecular mechanism(s) have been difficult to elucidate. A number of recent studies have determined the effect of exercise training on expression of a small number of selected genes, but the results of these studies have not yielded a consistent view and have not provided a mechanism for the described changes in contractile function (6, 16, 22, 39). We undertook an oligonucleo-
tide microarray analysis to determine the effects of exercise training on a large number of genes. With the use of our acceptance criteria, we found that 10 genes were increased and 17 genes were decreased in trained vs. control ventricular tissue. A number of the key changes are discussed below.

**Genes related to cardiac hypertrophy.** One of the most common cardiac adaptations to an endurance exercise training program is moderate cardiac hypertrophy. We found that exercise training increased heart mass by 14% over control. Previous reports have indicated that, under other hypertrophic stimuli such as pressure overload, a so-called “fetal gene program” is induced (24) that includes the downregulation of several cardiac proteins and the upregulation of genes such as β-myosin heavy chain (MHC), skeletal α-actin, atrial natriuretic factor (ANF), the GLUT1 glucose transporter, and aMLC-1 (10, 11, 19, 20, 24, 26, 30). Despite clear evidence of hypertrophy in response to the training program (Table 1), we found no evidence for the induction of any of these “fetal genes” with the exception of aMLC-1, which is discussed below. In fact, we found a decrease in the expression of several of these genes previously related to hypertrophy including ANF, skeletal α-actin, and the GLUT1 glucose transporter. Previous reports on the effect of training on the expression of ANF by ventricular tissue are in conflict. ANF mRNA levels were found to be increased by a rat swim training program (3), a dog treadmill training program (15), and a mouse voluntary wheel running program (1), but unchanged by a treadmill training program in rats (2, 12). Because of this uncertainty, we verified the results of our microarray analysis for ANF using RT-PCR and confirmed that ANF expression was decreased in trained compared with control tissue.

Interestingly, we did not observe an increase in the expression of the “fetal gene program” despite the
training-induced increase in the expression of IGF binding protein 3 that we observed in our microarray expression analysis. IGF binding protein 3 has been shown previously to be upregulated in a rodent model of heart disease (33), and increased IGF binding protein 3 was shown to induce transcription of ANF and β-MHC (9). We found an increase in IGF binding protein 3 expression with training but a decrease in ANF expression and no change in β-MHC expression.

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 is thought to initially be an adaptive response that temporarily augments or maintains cardiac output. However, this growth eventually results in decreased cardiac function. Cardiac hypertrophy as a result of exercise training is generally thought to improve cardiac function, and this improved function is maintained. As mentioned above, the changes in gene expression that we observed with exercise training are substantially different, in some cases opposite, than in hypertrophy associated with pressure overload or cardiac myopathy. This would suggest that these different hypertrophic stimuli operate by significantly different signaling pathways. However, there are clearly some similarities in the hypertrophic response to exercise training and pathological stimuli as evidenced by the increases in aMLC-1 and IGF binding protein 3 expression.

Contractile protein genes. Previous studies (6, 16, 18, 39) of training-induced changes in myocardial contractile function suggest the possibility of alterations in contractile protein expression as a result of exercise training. From this standpoint, the most important result of our expression analysis was the increase in the expression of the atrial isoform of MLC-1 in ventricular tissue from trained hearts. To confirm the microarray results, we performed RT-PCR using a primer specific for aMLC-1 and also tested for the presence of aMLC-1 protein using 2-D gel electrophoresis. Both of these methods confirmed that aMLC-1 expression is increased in ventricular tissue at both the mRNA and protein levels as a result of the endurance exercise training program.

Myosin is a hexameric protein consisting of two heavy chains and two pairs of light chains. In the adult mammalian ventricle, two isoforms of MHC are expressed: α and β. The young adult rat ventricle expresses predominantly the α-MHC isoform. As mentioned above, myocardial hypertrophy resulting from pressure overload results in an increased expression of β-MHC, but the effect of exercise training on cardiac MHC expression is less clear. A number of previous studies have suggested that exercise training induces an increase in α-MHC isoform expression (12, 23, 27). However, there are studies that have found no evidence for a change in MHC expression in response to exercise training (7, 36) as well as studies that have found evidence for a training-induced increase in β-MHC expression (8). Our expression analysis indicated no change in MHC isoform expression, but our results do show, for the first time, a training-induced change in MLC isoform expression.

There are two subfamilies of light chains associated with myosin: the essential light chains (ELC; also referred to as MLC-1 or MLC-3) and the RLC (also referred to as MLC-2). There are multiple isoforms of both ELC and RLC expressed in rat striated muscle (for a review, see Ref. 29). The expression of these MLC isoforms changes throughout development. The fetal rat heart expresses an embryonic form of the ELC that is identical to the aMLC-1 isoform. In the course of development, light chain expression changes in ventricular tissue to the ventricular isoform (vMLC-1), which is identical to the slow skeletal isoform of MLC-1. Thus, in the adult rat heart, atrial tissue expresses exclusively aMLC-1, whereas ventricular tissue expresses vMLC-1 (35).

This pattern of MLC-1 expression in ventricular tissue has been shown to change under pathological conditions. In both human hypertrophic cardiomyopathy (19) and in a porcine model of hypertension (20), aMLC-1 expression has been shown to be increased in ventricular myocardium. In failing human hearts, the amount of aMLC-1 ranged from 0% to 10.3% of the total MLC-1 pool (i.e., aMLC-1 + vMLC-1), whereas no aMLC-1 was detected in control human heart tissue (19). In porcine ventricular tissue, the aMLC-1 content increased from undetectable in control tissue to 16.9% of the total MLC-1 content in hypertrophied ventricles (20).

Expression of aMLC-1 in ventricular tissue has been shown to alter the contractile properties of the myocardium. Studies on pathological hypertrophy of human and porcine myocardium showed that the increase in aMLC-1 was associated with increased Ca$^{2+}$ sensitivity of tension (19) and increased maximal shortening velocity (20). The magnitude of these alterations in contractile properties were found to be significantly correlated with the magnitude of the increase in aMLC-1 expression, suggesting this change in MLC-1 isoform expression represents an underlying molecular mechanism for the changes in contractile function accompanying the pathological condition. Sanbe et al. (28) used a transgenic mouse model to overexpress ectopic MLC-1 isoforms and found that shortening velocity and power output were increased when aMLC-1 was expressed in ventricular tissue. The mechanism(s) for the effects of increased aMLC-1 expression to alter myocardial contractility is not known. It has been demonstrated that the NH$_2$-terminal region of the MLC-1 molecule interacts with the actin filament during cross-bridge formation (37). Sequence differences between aMLC-1 and vMLC-1 result in a difference in charge in this NH$_2$-terminal region that may affect the ability of the light chain to bind to actin (19).

These effects on myocardial contractile properties of increased aMLC-1 expression in ventricular myocardium are remarkably similar to alterations in contractile properties associated with exercise training. Previous studies have indicated that training increases the Ca$^{2+}$ sensitivity of tension in cardiac myocytes (6, 39) and increases the myocardial maximal shortening velocity and power.
output (16). These results suggest that the training-induced increase in αMLC-1 expression found in the present study may be an underlying molecular mechanism for previously observed contractile changes in response to endurance exercise training.

The results of our microarray analysis did not show a decrease in vMLC-1 isoform expression to accompany the increase in αMLC-1 expression. This is consistent with the previous findings of Buttrick et al. (3), who found no effect of exercise training on vMLC-1 expression. These data suggest that there may not be a shift in expression of MLC-1 isoforms, with αMLC-1 expression increased and vMLC-1 expression decreased, but instead an overexpression of αMLC-1 in ventricular tissue. The results of studies of hypertrophied myocardium (19, 20) as well as transgenic mouse studies of overexpression of MLC-1 isoforms (28) suggest that overexpression of αMLC-1 in ventricular myocardium results in stoichiometric incorporation of the αMLC-1 isoform into the myofilaments even in the absence of changes in expression of the vMLC-1 isoform.

Limitations to microarray analysis. Although oligonucleotide microarray analysis provides a powerful tool for determining changes in expression for a large number of genes, there are a number of important limitations to the interpretation of our results. First, the changes reported here represent only one time point in the process of myocardial adaptation to exercise, i.e., after 11 wk of progressive treadmill training. Other studies examining the effect of exercise training on cardiac gene expression have used training durations of 4 wk (1), 6 wk (3), 10 wk (2), 13 wk (12, 39), and 55 wk (15). It is likely that gene expression is altered along the entire time course of the training protocol, and the set of genes that we have identified represents only those genes with altered expression at this particular time point. Second, it is recognized that our ventricular samples are mixed tissue samples. It is likely that blood, neural tissue, and vascular tissue, as well as myocardial tissue, contributed to the total RNA pool on which expression analysis was done. Thus some of the gene expression changes that we have reported may not be adaptations of the myocardium per se. For example, the decrease in uncoupling protein 2 expression that we report here and others have reported in the heart (4) may be due to training-induced changes in uncoupling protein 2 expression in coronary vascular tissue (38).

In conclusion, we have shown that endurance exercise training induces altered the expression of a number of genes in the heart including increased expression of the atrial isoform of MLC-1 in ventricular tissue. Previous reports indicate that increased αMLC-1 content has effects on the contractile properties of ventricular myocardial preparations similar to those seen with adaptation to exercise training. Thus we conclude that increased αMLC-1 expression in ventricular myocardium represents a possible molecular mechanism for the previously observed changes in myocardial contractile function associated with exercise training.

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EXERCISE INCREASES ATRIAL MYOSIN LIGHT CHAIN EXPRESSION

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