Am J Physiol Heart Circ Physiol 284: H830–H837, 2003.
First published November 7, 2002; 10.1152/ajpheart.00761.2002.

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

GARY M. DIFFEE,[1] ERIC A. SEVERSEN,[1] THOR D. STEIN,[2,3] AND JEFFREY A. JOHNSON[2,3,4,5]
[1]*Department of Kinesiology,* [2]*School of Pharmacy,* [3]*Center for Neuroscience,* [4]*Molecular and Environmental Toxicology Center, and* [5]*Waisman Center, University of Wisconsin, Madison, Wisconsin 53706*

Submitted 30 August 2002; accepted in final form 28 October 2002

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 Ca^{2+} 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 (aMLC-1) in the trained ventricular tissue. We confirmed microarray results for aMLC-1 using RT-PCR and also confirmed a training-induced increase in aMLC-1 protein using two-dimensional gel electrophoresis. aMLC-1 content has been previously shown to be increased in human cardiac hypertrophy and has been associated with increased Ca^{2+} sensitivity of tension and increased power output. These results suggest that increased expression of aMLC-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 Ca^{2+} sensitivity of tension was increased as a result of training in permeablized 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 permeablized 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 Ca^{2+} 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

Address for reprint requests and other correspondence: G. M. Diffee, Biodynamics Laboratory, Univ. of Wisconsin, Dept. of Kinesiology, 2000 Observatory Drive, Madison, WI 53706 (E-mail: gmdiffee@facstaff.wisc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

 0363-6135/03 $5.00 Copyright © 2003 the American Physiological Society

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 MLC 1 (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 Ca^{2+}-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 Tissumizer in TRIzol reagent (Invitrogen), and total RNA was extracted. An RNEasy 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-$(dT)_{24}$ primer incorporating a T7 RNA polymerase promoter (Integrated DNA Technologies). cRNA was prepared and biotin 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-phycoerythrin using a fluidics station. The probe arrays were scanned at 3-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 the difference calls, and the values varied from −18 to 18. The cutoff value for increase or decrease was set as $\pm n^2$ ($n = 3$) 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 8 and not be on the list. Therefore, the cutoff in the 3×3 matrix would be $n^2 = 9/-9$ (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 AT-3′ and 5′-CCA GTA TGA GTC CAG TGC TC-3′) and atrial natriuretic peptide (ANP; 5′-TTC AAG AAC CTG CTA GAC CA-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 $MgCl_2$, 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 by 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

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

	Control	Trained
Body weight, g		
Before training program	163.5 ± 1.7	160.5 ± 1.8
After training program	275.6 ± 4.3	267.7 ± 2.3
Heart weight, mg	736 ± 15	790 ± 14*
Heart weight/body weight, mg/g	2.68 ± 0.04	2.95 ± 0.05*
Plantaris citrate synthase activity, $\mu mol \cdot min^{-1} \cdot g\ wet\ wt^{-1}$	15.5 ± 1.7	22.6 ± 1.8*

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

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 Protean isoelectric focusing cell and 11-cm precast immobilized pH gradient (pI) gel strips (pH 3–10). Protein (300 μg) in sample buffer plus 1.85% CHAPS and 0.185% carrier ampholytes were loaded onto the strips via 1 h of passive rehydration and 12 h of active loading at 50 V and 20°C. The Bio-Rad IEF unit was programmed to rapidly ramp to 250 V for the first 15 min, rapidly ramp to 6,700–7,000 V for the next 2.5 h (limited to 50 mA/strip), and hold at peak voltage and 50 mA/strip for 35,000 V·h. Strips were held at 500 V at the conclusion of their run until removed from the power unit. Strips were incubated in equilibration *buffer I* [125 mM Tris·HCl (pH 6.8), 20% glycerol, 2% SDS, 6 M urea, and 2% DTT] and *buffer II* [125 mM Tris·HCl (pH 6.8), 20% glycerol, 2% SDS, 6 M urea, and 2.5% iodoacetamide] for 20 min each. After equilibration of the strips, second-dimension PAGE was performed using 12.5% Bio-Rad Criterion precast gels with IPG + one-well combs, run at 20 mA/gel for 45 min and 30 mA/gel for the duration of the run (2.5 h total). Gels were stained 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 locations of the aMLC-1 and ventricular MLC-1 (vMLC-1) spots were determined based on the predicted isoelectric point and molecular mass of these proteins as well as by comparison to previously published 2-D gel analysis of these proteins (20, 25).

Citrate synthase assay. Plantaris muscles were removed immediately after excision of the heart. The muscles were trimmed of connective tissue, quick frozen in liquid nitrogen, and stored at −80°C. The plantaris was thawed and homogenized in a potassium phosphate buffer (pH 7.4) and assayed for citrate synthase activity at 25°C as previously described (32).

Solutions. The relaxing solution used during heart removal has been described previously (6) and contained 100 mM KCl, 1.75 mM EGTA, 10 mM imidazole, 4 mM ATP, and 5 mM $MgCl_2$, adjusted to pH 7.0 with KOH.

Statistical analysis. Data for all experiments except microarray analysis are presented as means ± SD. Between-group comparisons (trained vs. control) were made using Student's *t*-test, with $P < 0.05$ considered to indicate a statistically significant difference.

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*

Gene Description	R	FC	CV
Decreases			
Uncoupling protein 2	−18	−3.1 ± 0.25	0.08
Monoamine oxidase A	−18	−2.5 ± 1.05	0.42
Decorin	−18	−1.7 ± 0.82	0.48
Eukaryotic translation factor $1_{\alpha 2}$	−18	−3.2 ± 1.75	0.55
Na^+-K^+-ATPase, α_1-subunit	−14	−1.8 ± 0.33	0.18
Skeletal muscle α-actin	−14	−2.2 ± 1.21	0.55
Heat shock protein 70	−14	−2.8 ± 1.10	0.39
Pyruvate dehydrogenase phosphatase enzyme	−12	−4.3 ± 1.33	0.21
GLUT1 glucose transporter	−12	−2.0 ± 1.07	0.54
γ-Atrial natriuretic peptide precursor	−12	−1.9 ± 0.48	0.25
Cardionatrin precursor	−12	−2.3 ± 0.53	0.13
Atrial natriuretic factor	−12	−2.2 ± 1.4	0.64
Glutamine synthetase	−10	−3.3 ± 0.87	0.26
Calreticulin	−10	−2.7 ± 1.16	0.43
Phosphotidylinositol transfer protein	−10	−1.5 ± 0.94	0.63
Dihydrolipoamide acetyltransferase	−10	−1.6 ± 0.46	0.29
Plasma glutathione peroxidase	−10	−2.1 ± 1.22	0.58
Increases			
IGF binding protein 3	18	4.2 ± 0.34	0.09
VEGF receptor 2/FLK-1	16	2.4 ± 0.95	0.40
Atrial myosin light chain 1	16	2.7 ± 1.12	0.41
β-Globin gene	14	1.7 ± 0.73	0.43
Laminin chain-β_2	14	3.0 ± 1.05	0.35
β-Actin	12	2.2 ± 1.26	0.57
Glutathione-*S*-transferase	12	1.6 ± 0.67	0.42
Cytochrome *P*-450	10	2.5 ± 0.73	0.29
Rat pancreatitis-associated protein	10	2.3 ± 1.36	0.28
2,4-Dienoyl-CoA reductase	10	1.5 ± 0.42	0.28

Genes that were significantly increased or decreased in trained compared with control samples are shown. R, ranking from 3 × 3 comparison as described 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. 2*B*. 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 $n = 6$ 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 ($P < 0.05$).

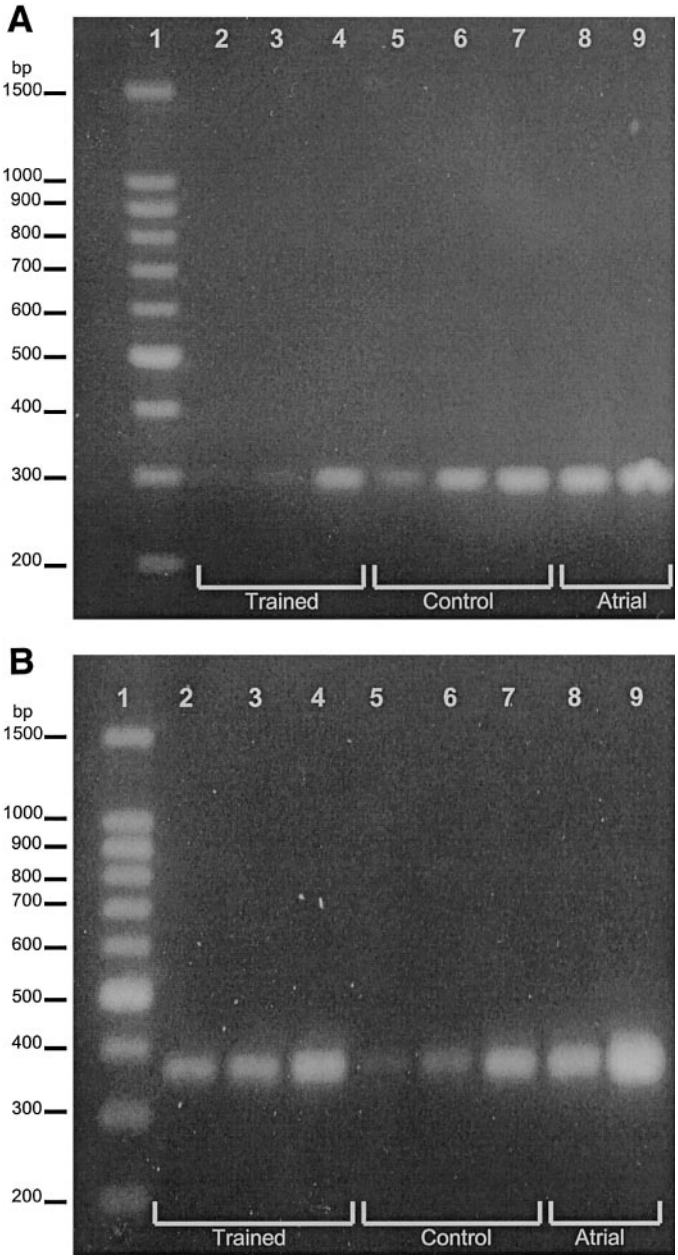

Fig. 1. Results of RT-PCR analysis of expression of atrial natriuretic peptide (ANP; *A*) and atrial myosin light chain 1 (aMLC-1; *B*). For both gels, *lane 1* is a DNA marker; *lanes 2*, *3*, and *4* are RT-PCR products using RNA isolated from trained ventricular tissues; and *lanes 5*, *6*, and *7* are products from control ventricular tissue. The three samples in each group are from 21 (*lanes 2* and *5*), 24 (*lanes 3* and *6*), and 27 cycles (*lanes 4* and *7*) of the PCR. Products from atrial tissue (*lanes 8* and *9*) were run for 21 and 24 cycles.

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-

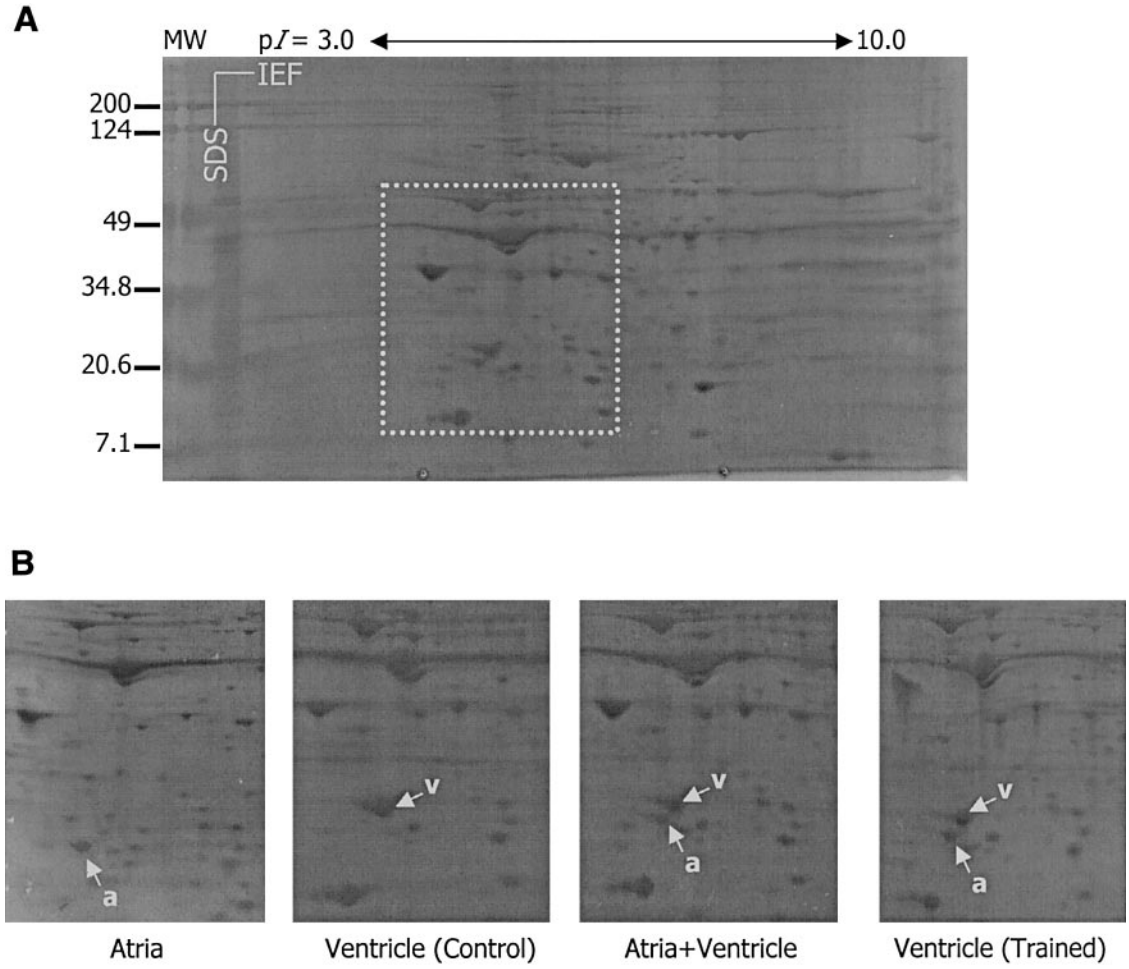

Fig. 2. Results of two-dimensional (2-D) electrophoresis analysis of aMLC-1 protein expression in trained and control ventricular tissue. *A*: whole gel results of first-dimension isoelectric focusing using a pH 3–10 gradient and second-dimension SDS-PAGE with a 12.5% acrylamide gel. *B*: closeup of the aMLC-1/ventricular MLC-1 (vMLC-1) region of gels (box in *A*) used for analysis. Shown are representative gels using homogenates from control atrial tissue, control ventricular tissue, a mixture of atrial and ventricular homegenates, and trained ventricular tissue. a, aMLC-1; v, vMLC-1. Identification of aMLC-1 and vMLC-1 is based on predicted isoelectric point (pI) and molecular weight (MW) values for these proteins as well as previously published 2-D electrophoretic analyses of these proteins.

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 adult isoforms 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 aMLC-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 aMLC-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 aMLC-1 expression increased and vMLC-1 expression decreased, but instead an overexpression of aMLC-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 aMLC-1 in ventricular myocardium results in stoichiometric incorporation of the aMLC-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 incoupling 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 aMLC-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 aMLC-1 expression in ventricular myocardium represents a possible molecular mechanism for the previously observed changes in myocardial contractile function associated with exercise training.

We thank Helga Ahrens and Dr. Santhanum Swaminathan in the Cell Markers Facility and Services Core of the University of Wisconsin-Madison Environmental Health Services Center for expert assistance with the 2-D electrophoresis experiments.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-61410 (to G. M. Diffee).

REFERENCES

1. **Allen DL, Harrison BC, Maass A, Bell ML, Byrnes WC, and Leinwand LA.** Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. *J Appl Physiol* 90: 1900–1908, 2001.
2. **Azizi C, Bouissou P, Galen FX, Lattion AL, Lartigue M, and Carayon A.** Alterations in atrial natriuretic peptide gene expression during endurance training in rats. *Eur J Endocrinol* 133: 361–365, 1995.
3. **Buttrick PM, Kaplan M, Leinwand LA, and Scheuer J.** Alterations in gene expression in the rat heart after chronic pathological and physiological loads. *J Mol Cell Cardiol* 26: 61–67, 1994.
4. **Boss O, Samec S, Desplanches D, Mayet M, Seydoux J, Muzzin P, and Giacobino J.** Effect of endurance training on mRNA expression of uncoupling proteins 1, 2, and 3 in the rat. *FASEB J* 12: 335–339, 1998.
5. **Diffee GM and Nagle DR.** Exercise training alters the length dependence of contractile properties in rat myocardium. *J Appl Physiol*. In press.
6. **Diffee GM, Seversen EA, and Titus MM.** Exercise training increases the Ca^{2+} sensitivity of tension in rat cardiac myocytes. *J Appl Physiol* 91: 309–315, 2001.
7. **Farrar RP, Starnes JW, Cartee GD, Oh PY, and Sweeney L.** Effect of exercise training on cardiac isozyme composition during the aging process. *J Appl Physiol* 64: 880–883, 1988.
8. **Fitzsimons DP, Bodell PW, Herrick RE, and Baldwin KM.** Left ventricular functional capacity in the endurance-trained rodent. *J Appl Physiol* 69: 309–312, 1990.
9. **Henson M, Damm D, Lam A, Garrad LJ, White T, Abraham JA, Schreiner GF, Stanton LW, and Joly AH.** Insulin-like growth factor-binding protein-3 induces fetalization in neonatal rat cardiomyocytes. *DNA Cell Biol* 19: 757–763, 2000.
10. **Izumo S, Lompre A, Matsuoka R, Koren G, Schwartz K, Nadal-Ginard B, and Mahdavi V.** Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy. *J Clin Invest* 79: 970–977, 1987.
11. **Izumo S, Nadal-Ginard B, and Mahdavi V.** Protooncongene induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci USA* 85: 339–343, 1988.
12. **Jin H, Yang R, Li W, Lu H, Ryan A, Ogasawara A, Van Peborgh J, and Paoni N.** Effects of exercise on cardiac function, gene expression and apoptosis in rats. *Am J Physiol Heart Circ Physiol* 279: H2994–H3002, 2000.
13. **Li J and Johnson JA.** Time-dependent changes in antioxidant responsive element (ARE)-driven gene expression through use of a simple noise-filtering process for microarray data. *Physiol Genomics* 9: 137–144, 2002.
14. **Li J, Lee JM, and Johnson JA.** Microarray analysis reveals an antioxidant responsive element-driven gene set involved in conferring protection from an oxidative stress-induced apoptosis in IMR-32 cells. *J Biol Chem* 277: 388–394, 2002.
15. **Mantymaa P, Arokoski J, Porsti I, Perhonen M, Arvola P, Helminen H, Takala TES, Leppaluoto J, and Ruskoaho H.** Effect of endurance training on atrial natriuretic peptide gene expression in normal and hypertrophied hearts. *J Appl Physiol* 76: 1184–1194, 1994.
16. **Molè P.** Increased contractile potential of papillary muscles form exercised-trained rat hearts. *Am J Physiol* 234: 421–425, 1978.
17. **Moore RL and Korzick DH.** Cellular adaptations of the myocardium to chronic exercise. *Prog Cardiovasc Dis* 37: 371–396, 1995.
18. **Moore RL, Musch TI, Yelamarty RV, Scaduto RC Jr, Semanchick AM, Elensky M, and Cheung JY.** Chronic exercise

alters contractility and morphology of isolated rat cardiac myocytes. *Am J Physiol Cell Physiol* 264: C1180–C1189, 1993.
19. **Morano I, Hadicke K, Haase H, Bohm M, Erdmann E, and Schaub MC.** 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.
20. **Morano M, Boels P, Haworth SG, Haase H, and Morano I.** 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 Sugi H and Pollock GH. New York: Plenum, 1998, p. 481–488.
21. **Nassar R, Malouf NN, Kelly MB, Oakley AE, and Anderson PAW.** Force-pCa relation and troponin T isoforms of rabbit myocardium. *Circ Res* 69: 1470–1475, 1991.
22. **Natali AJ, Wilson LA, Peckham M, Turner DL, Harrison SM, and White E.** Different regional effects of voluntary exercise on the mechanical and electrical properties of rat ventricular myocytes. *J Physiol* 541: 863–875, 2002.
23. **Pagani ED and Solaro RJ.** Swimming exercise, thyroid state, and the distribution of myosin isoenzymes in rat heart. *Am J Physiol Heart Circ Physiol* 245: H713–H720, 1983.
24. **Parker T, Packer S, and Schneider M.** Peptide growth factors can provoke "fetal" contractile protein gene expression in rat cardiac myocytes. *J Clin Invest* 85: 507–514, 1990.
25. **Pleiβner KP, Regitz-Zagrosek V, Weise C, Neuβ M, Krudewagen B, Soding P, Buchner K, Hucho F, Hildebrandt A, and Fleck E.** Chamber specific expression of human myocardial proteins detected by two-dimensional gel electrophoresis. *Electrophoresis* 16: 841–850, 1995.
26. **Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, and Taegtmeyer H.** Metabolic gene expression in fetal and failing human heart. *Circulation* 104: 2929–2931, 2001.
27. **Rupp H.** The adaptive changes in the isoenzyme pattern of myosin from hypertrophied rat myocardium as a result of pressure overload and physical training. *Basic Res Cardiol* 76: 79–88, 1981.
28. **Sanbe A, Gulick J, Hayes E, Warshaw D, Osinska H, Chan CB, Klevitsky R, and Robbins J.** Myosin light chain replacement in the heart. *Am J Physiol Heart Circ Physiol* 279: H1355–H1364, 2000.
29. **Schiaffino S and Reggiani C.** Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol Rev* 76: 371–423, 1996.
30. **Schwartz K, de la Bastie D, Bouveret P, Oliviero P, Alonso S, and Buckingham ME.** α-Skeletal actin mRNAs accumulate in hypertrophied adult rat hearts. *Circ Res* 59: 551–555, 1986.
31. **Solaro RJ, El-Saleh SC, and Kentish JC.** Ca^{2+}, pH and the regulation of cardiac myofilament force and ATPase activity. *Mol Cell Biochem* 89: 163–167, 1989.
32. **Srere PA.** Citrate synthase. *Methods Enzymol* 13: 3–5, 1969.
33. **Stanton LW, Garrad LJ, Damm D, Garrick BL, Lam A, Kapoun AM, Zheng Q, Protter AP, Schreiner GF, and White RT.** Altered patterns of gene expression in response to myocardial infarction. *Circ Res* 86: 939–945, 2000.
34. **Sweeney HL and Stull JT.** Phosphorylation of myosin in permeabilized mammalian cardiac and skeletal muscle cells. *Am J Physiol Cell Physiol* 250: C657–C660, 1986.
35. **Swynghedauw B.** Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. *Physiol Rev* 66: 710–771, 1986.
36. **Tibbits GF, Barnard RJ, Baldwin KM, Cugalj N, and Roberts NK.** Influence of exercise on excitation-contraction coupling in rat myocardium. *Am J Physiol Heart Circ Physiol* 240: H472–H480, 1981.
37. **Trayer IP, Trayer HR, and Levine BA.** Evidence that the N-terminal region of the A1-light chain of myosin interacts with the C-terminal region of actin. *Eur J Biochem* 164: 259–266, 1987.
38. **Wheeler A and LJ Rubin.** UCP2 expression in liver and coronary artery of exercise trained normal and hyperlipidemic swine (Abstract). *J Mol Cell Cardiol* 34: A5, 2002.
39. **Wisloff U, Loennechen JP, Falck G, Beisvag V, Currie S, Smith G, and Ellingsen O.** Increased contractility and calcium sensitivity in cardiac myocytes from endurance trained rats. *Cardiovasc Res* 50: 495–508, 2001.
40. **Wolska BM, Keller RS, Evans CC, Palmiter KA, Phillips RM, Muthuchamy M, Oehlenschlager J, Wieczorek DF, de Tombe PP, and Solaro RJ.** Correlation between myofilament response to Ca^{2+} and altered dynamics of contraction and relaxation in transgenic cardiac cells that express beta-tropomyosin. *Circ Res* 84: 745–751, 1999.
41. **Zhang R, Zhao J, Mandveno A, and Potter JD.** Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation. *Circ Res* 76: 1028–1035, 1995.