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Proteomic Analysis Reveals Late Exercise Effects on Cardiac Remodeling Following Myocardial Infarction

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Abstract

Exercise has been shown to improve function of the left ventricle (LV) following myocardial infarction (MI). The mechanisms to explain this benefit have not been fully delineated, but may involve improved mechanics resulting in unloading effects and increased endothelial nitric oxide synthase levels.[1,2] Accordingly, the goal of this study was to determine how the LV infarct proteome is altered by a post-MI exercise regimen. Sprague-Dawley rats underwent ligation of the left descending coronary artery to induce MI. Exercise training was initiated four weeks post-MI and continued for 8 weeks in n=12 rats. Compared with the sedentary MI group (n=10), the infarct region of rats receiving exercise showed 20 protein spots with altered intensities in two-dimensional gels (15 increased and 5 decreased; p<0.05). Of 52 proteins identified in 20 spots, decreased levels of voltage-dependent anion-selective channel 2 and increased levels of glutathione peroxidase and manganese superoxide were confirmed by immunoblotting. Cardiac function was preserved in rats receiving exercise training, and the beneficial effect was linked with changes in these 3 proteins. In conclusion, our results suggest that post-MI exercise training increases anti-oxidant levels and decreases ion channel levels, which may explain, in part, the improved cardiac function seen with exercise.

Keywords

myocardial infarction; exercise; proteomics; anti-oxidant; two-dimensional gel electrophoresis; left ventricular remodeling

Introduction

Remodeling of the left ventricle (LV) following myocardial infarction (MI) remains a significant clinical problem.[3] While 30 day survival post-MI has dramatically improved

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over the last 30 years, the number of MI patients who progress to adverse remodeling and congestive heart failure approaches 25%. [4] Exercise is one post-MI therapeutic option that has been evaluated in both humans and animal models, and has been shown to improve outcomes. [5]

In humans, previous studies have shown that exercise training post-MI has favorable effects on LV remodeling and improved LV functional capacity, ejection fraction, and early LV diastolic filling. [6] Furthermore, exercise increases antioxidant activity in the heart. Increased manganese superoxide dismutase in skeletal muscle has been strongly linked to exercise, but there are also reports that glutathione peroxidase and catalase are induced with exercise training. [7] While the effect of exercise on post-MI remodeling is complicated, not all patients seem to benefit equally from exercise post-MI and the effects appear to be context dependent. [1,2] The timing of when to start the exercise regimen is controversial, in part due to the different experimental designs used, which include different infarct size groups and different exercise programs that vary in mode (swimming vs running), [8] intensity, duration, and the course of training. All of these differences among experiments may contribute to the different findings.

Our group has compared exercise training started one week and six weeks after MI in rats and found that exercise training affected LV remodeling and function similarly in the two exercise groups. [9] We have previously reported the cardioprotective effects of exercise training post-MI specifically at the molecular and cellular level in rats, showing that early exercise training post-MI reduces the expression of endogenous tissue inhibitors of metalloproteinases (TIMPs) at the gene and protein levels, improves the balance between matrix metalloproteinases (MMPs) and TIMPs, lowers the expression of angiotension converting enzyme (ACE) and angiotension II receptor type I (AT1), significantly reduces total collagen content, and, importantly, improves post-MI LV function. [5] Other teams have also reported benefits of exercise in rat infarct models. [10,11] The Li lab has reported that hsp20 increases in the rat cardiac proteome with exercise, [10] and Burniston has shown that creatine kinase decreases with exercise. [11] However, a proteomic evaluation of post-MI infarct tissue from exercised rats has not been made, to determine global changes in protein expression that result from exercise.

Accordingly, this study was designed to evaluate the LV infarct proteome of post-MI rats, comparing an MI group that exercised for 8 weeks to a sedentary control MI group. We hypothesized that the exercise group would demonstrate an altered infarct proteome, consistent with past reports of improved LV function with exercise.

Methods

2.1 Rat model of myocardial infarction

The rats used in these experiments were treated in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the study protocols were approved by the Institutional Animal Care and Use Committee at the University of Texas at San Antonio. All animal experiments met the *International Guiding Principles for Biomedical Research Involving Animals*. Seven-week-old male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA) were housed at constant temperature ($22\pm 2^{\circ}\text{C}$) on a 12 h light/dark cycle. They were fed *ad libitum* on standard laboratory rat chow and had free access to tap water. MI was made by permanent ligation of the left anterior descending coronary artery as described previously. [5]

2.2 Experimental design

The experimental design is shown in Figure 1. At four weeks post-MI, cardiac function was evaluated by echocardiography, and the group was randomly divided into two subgroups that showed similar MI size and post-MI functional parameters. The two groups were sedentary MI (MI-Sed; n=10), and MI plus exercise (MI-Ex; n=12). In addition, sham-operated (Sham-Sed) and sham-operated plus exercise (Sham-Ex) control rats were included in the functional measurements.

MI-Ex rats started exercising at 4 weeks post-MI using a motorized rodent treadmill, and exercise was continued for 8 weeks. The Sham-Ex group also exercised for 8 weeks, whereas the two sedentary groups remained sedentary throughout the experiment period. To allow gradual adaptation to exercise and avoid stress, exercise training was initiated at 10 m/min, 5° incline for 10 min per session. The speed and duration were gradually increased to 16 m/min and 50 min per session (including a 5 min warm-up at 10 m/min) and maintained constant throughout the experiment. Exercise training was performed 5 days a week for 8 weeks. The determination of the exercise regimen was based on previous studies from one of our laboratories.[5,9] This exercise regiment improved aerobic capacity of post-MI rats, as indicated by elevated muscle citrate synthase activity, without affecting mortality rates.[9] The exercise intensity was moderate and 55-60% of maximal oxygen consumption.[12-14]

2.3 Echocardiographic measurements

Echocardiographic measurements were performed on the day before the initiation of post-MI exercise training (4 weeks post-MI), and after 8 weeks of exercise training (12 weeks post-MI). We used an ultrasound system equipped with a 10 MHz transducer (SonoHeart Elite, SonoSite Bothell, WA, USA). Rats were anaesthetized with isoflurane (0.5-2%) mixed with oxygen, and a two-dimensional short-axis view of the LV was obtained at the level of the papillary muscle to record M-mode tracings. We measured LV anterior wall thickness; LV end-diastolic and LV end-systolic volumes; stroke volume; cardiac output (SV × heart rate); and ejection fraction. All measurements were averaged over three consecutive cardiac cycles. Because body weights were decreased in the exercised groups, measurements were normalized to body weight.

2.4 Protein extraction

At sacrifice, the infarct regions were separated from the remote regions (based on wall thinning and free wall location) and the infarct regions were used for downstream proteomic analysis. Control sham LV samples were not compared to the infarct samples, because cell and tissue composition is much different with MI. To avoid confounding issues of examining myocyte-depleted to myocyte-rich regions, the sham animals were used to establish baseline cardiac function values and were not used in the proteomic analysis. The proteomic comparison examined was between infarct regions from the sedentary and exercise groups. The infarct regions of the LVs from the two MI groups were homogenized in extraction buffer (0.8 mL protein extraction reagent 4 per 50mg tissue; Sigma. Reagent 4 contains 7 M urea, 2 M thiourea, 40 mM Trizma® base and the detergent 1% C7BzO) and 1× Complete Protease Inhibitor Cocktail (Roche). The protein concentration of each sample was determined by the Bradford assay, and beta-actin immunoblotting was performed to verify equal protein amounts among the samples before evaluation by two-dimensional gel electrophoresis.

2.5 Two-dimensional gel electrophoresis (2-DE)

For each biological replicate, there were n=3 analytical replicates for the 2-DE gel analysis. Protein extracts (500 µg; n=10 for MI-Sed and n=12 for MI-Ex groups) were reduced in

2.5% tributylphosphine containing 1× Complete Protease Inhibitor Cocktail (Roche) at room temperature for 1 h. Iodoacetamide was added to a final concentration of 3% and the samples were incubated at room temperature for 1h. The samples were then centrifuged at $425 \times g$ for 5 min to pellet debris. Supernatants were harvested and acetone was added to a final concentration of 80%; proteins were precipitated at room temperature for 30 min. The samples were centrifuged at $20,817 \times g$ for 10 min and supernatants were discarded. The pellets were air-dried and resuspended in 200 μ l of extraction buffer and 1× Complete Protease Inhibitor Cocktail. After incubation at 30°C for 30 min, 500 μ g of each sample was loaded to an 11-cm pH 3 – 10 IPG strip (Proteome Systems), rehydrated overnight at room temperature, and then focused for 75,000 Vh. The focused-IPG strips were then equilibrated in 5 ml of Equilibration Buffer (Proteome Systems) for 20 min. Equilibrated strips were loaded on pre-cast gels (Criterion™ XT 4-12% Bis-Tris, 11cm, 1mm; Bio-Rad) for the second-dimension of electrophoresis. The gels were run at 200 V (25 – 50 mA/gel), fixed in 25% methanol/10% acetic acid at room temperature for 30 min, and stained overnight with the ProteomIQ™ Blue Gel Stain Kit (Proteome Systems). After staining the gels were scanned using a Kodak Image Station 4000MM camera interfaced with Molecular Imaging Software, version 4.0 (Eastman Kodak Company). Images were saved as 16-bit tiff files. The images were analyzed using the Progenesis PG240 software package (Nonlinear Dynamics). Using the PG240 software, we performed image quality control to optimize image capture, image alignment to match spots, and pre-filter to remove artifacts (e.g., damage or noise areas). The volume of an individual spot, the integrated intensity within the spot area, was normalized to the total spot volume of the whole gel to give a normalized spot volume of the spot. Spots that exhibited significant differences between the two groups ($p < 0.05$) were selected for protein identification by mass spectrometry.

2.6 Identification of spots by mass spectrometry

Gel spots were manually excised using a One Touch™ 1.5 mm 2-DE spot picker (The Gel Company) and digested *in situ* with trypsin (Promega modified) in 40 mM NH_4HCO_3 at 37°C for 4 h. Digests were analyzed by capillary HPLC-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) on a Thermo Fisher LTQ linear ion trap mass spectrometer fitted with a New Objective PicoView 550 nanospray interface. On-line HPLC separation of the digests was achieved with an Eksigent NanoLC micro HPLC: column, PicoFrit™ (New Objective; 75 μ m i.d.) packed to 10 cm with C18 adsorbent (Vydac; 218MSB5, 5 μ m, 300 Å); mobile phase A, 0.5% acetic acid (HAc)/0.005% trifluoroacetic acid (TFA); mobile phase B, 90% acetonitrile/0.5% HAc/0.005% TFA; gradient 2 to 42% B in 30 min; flow rate, 0.4 μ l/min. The following MS conditions were used: ESI voltage, 2.9 kV; isolation window for MS/MS, 3; relative collision energy, 35%; scan strategy, survey scan followed by acquisition of data dependent collision-induced dissociation (CID) spectra of the seven most intense ions in the survey scan above a set threshold. The uninterpreted CID spectra were searched using Mascot (Matrix Science) against the NCBI nr_20070216 database. Peak lists were created using extract_msn.exe. Trypsin was specified as the proteolytic enzyme and up to two missed cleavages were allowed. The precursor and fragment ion mass tolerances were both set at ± 0.8 Da. Methionine oxidation and cysteine carbamidomethylation were considered as variable modifications for all searches. Cross correlation of the Mascot results with X! Tandem and determination of protein identity probabilities was accomplished with Scaffold™ (Proteome Software).

2.7 Immunoblotting

Proteins selected for further immunoblot analysis were selected if an antibody was commercially available and 1) the protein was previously associated with exercise effects in skeletal or cardiac muscle or 2) the protein has not been previously described in the post-MI LV. To confirm changes in specific proteins, immunoblotting was performed using anti-rat

antibodies at the following dilutions: 14-3-3-zeta (Abcam, ab51129, 1:1,000); creatine kinase mitochondrial 2 (Abcam, ab55963, 1:1,000); glutathione peroxidase 1 (Abcam, ab21966, 1:10,000); manganese superoxide dismutase (MnSOD, Stressgen, SOD-111, 1:6,000); transgelin (Santa Cruz, sc-50446, 1:500); and voltage-dependent anion-selective channel 2 (VDAC2, Abcam, ab47104, 1:1000). For every sample, 10 µg of total protein was loaded in one lane of a 26-well 4–12% Criterion Bis-Tris gel (Bio-Rad). The secondary antibodies used were goat anti-rabbit IgG (Vector, PI-1000) or anti-sheep IgG₁ (Upstate, 12-342) used at 1:5000 dilution. Detection was performed using SuperSignal WestPico chemiluminescent substrate (Thermo Scientific, 34080). The blots were imaged using the 4000MM Kodak Image Station and the Molecular Imaging Software, version 4.0 (Kodak) was used for image densitometry analysis. Every blot was checked to confirm that no band was saturated and that the signal was in the dynamic range. Equal loading and transfer was confirmed by staining the membrane with MemCode™ Reversible Protein Stain (Thermo Scientific, 24580). Densitometry of the 42 kDa stained band was used to normalize the immunoblotting densitometries. Equal loading was also confirmed by β-actin immunoblotting. Densitometry values for the groups were 12093±530 unit for the MI-Sed group and 11963±824 units for the MI-Ex group (p=0.90).

2.8 Statistical analyses

Data are reported as mean ± SEM. All samples were analyzed individually and were not pooled at any step. Echocardiographic parameters were analyzed by ANOVA with Student Newman Keuls post-test to compare the 4 groups. Normalized spot intensities of 2-DE gels and normalized intensities for the immunoblots between MI-Sed and MI-Ex groups were analyzed by an unpaired Student's t-test. A p<0.05 was considered significant.

3. Results

3.1 Echocardiography analysis

Table 1 displays the characteristics of the animals in the study. The body weights in the two exercise groups were significantly lower compared to the two sedentary groups, consistent with an endurance exercise protocol. The infarct size between the MI-Sed and the MI-Ex rats was comparable. The heart weight to body weight ratio was significantly higher in the two MI groups, compared to the respective sham controls. Heart weight to body weight ratios were higher in the MI-Ex group when compared to the MI-Sed group, consistent with hypertrophic growth from exercise training. By echocardiography, the ratio of anterior wall thickness to body weight was similar between the MI-Sed and the MI-Ex groups. Those two groups, however, were statistically significant when compared to their respective sham counterparts. EDV and ESV were both increased in the MI groups. EDV increased to a greater extent in the MI-Ex group when compared to MI-Sed. Both EF and SV increased significantly in the rats that underwent exercise training post-MI, compared to MI-Sed values.

3.2 Identification of proteins using 2-DE Gels and mass spectrometry

From image analysis of the 2-DE gels, we detected 20 spots that showed significant differences in intensity when comparing the two MI groups (sedentary versus exercise). Out of the 20 spots, 5 spots (25%) were higher and 15 spots (75%) were lower in intensity in the exercise group compared to the sedentary group (Table 2). A total of 52 proteins were identified in the 20 spots, for an average of 2.6 proteins per spot. Proteins identified by mass spectrometry include lumican, 14-3-3, voltage dependent anion channel, transgelin, glutathione peroxidase, and manganese superoxide dismutase. A listing of the proteins identified is shown in Table 2, and the complete list with mass spectrometry details can be found in the supplementary Table 1.

3.3 Immunoblotting analysis to determine protein levels in post-MI LV

As an additional method to evaluate changes in protein expression, we performed immunoblotting analysis on the two MI sample groups using antibodies specific for six of the proteins identified. Of the six proteins analyzed, differences in the levels of one protein (voltage dependent anion channel 2) agreed with the 2-DE gel results (both methods showed decreased intensity). For two of the proteins (glutathione peroxidase and manganese superoxide dismutase), the gel spot showed decreased intensity in the MI-Ex group while the immunoblots showed increased intensity. For three proteins (creatine kinase mitochondrial 2, transgelin, and 14-3-3 zeta), the immunoblot results showed no difference in intensity between the two groups. Table 3 summarizes the agreement between results observed by 2-DE gel analysis and immunoblotting.

4. Discussion

The objective of this study was to use a proteomic approach to identify proteins altered in the infarct region of post-MI rats following an endurance exercise regimen. The most significant findings of this study were: 1) LV function improved in the exercise group post-MI, even when exercise was initiated four weeks post-MI; 2) VDAC 2 decreased; and 3) and GPX 1 and MnSOD increased in the of the exercise group compared with the sedentary MI group. Together, our results suggest that oxidative stress may be a chronic stimulus to induce adverse remodeling following MI and demonstrate that a primary benefit of post-MI exercise is the increase of anti-oxidant levels.

Our echocardiography data shows exercise post-MI improves LV function. Stroke volume, cardiac output, and ejection fraction all increased post-MI in the exercise group when compared to sedentary rats post-MI. This result agrees with previous literature.[5] Our results also show that exercise training increases end systolic and diastolic volumes post-MI, consistent with results shown by the Yamaguchi laboratory.[15] This group showed that dimensions and cardiac function was significantly increased in aging rats that had undergone a training regimen for 8 weeks.

Another interesting finding in our study was that exercise post-MI showed significantly decreased levels of voltage dependent anion channel 2 (VDAC 2). VDAC 2 is a protein located on the outer membrane of mitochondria and has gained increased interest due to its role in apoptosis and cancer.[16] Our team has previously reported that VDAC 2 levels increase in the LV of mice from middle age to old age, indicating that VDAC 2 may regulate some components of cardiac aging.[17] Of the different isoforms, VDAC 2 has been linked to the bcl family of proteins by both a BAK-dependent and –independent mechanism, and has been linked to other apoptotic molecules by means of signal transduction.[18,19] VDAC evaluation in the post-MI LV, however, is limited. Imahashi et al found that overexpression of Bcl-2 reduced ischemia/reperfusion injury through a direct interaction with VDAC.[18] Baines et al demonstrated that the coupling of protein kinase C ϵ with VDAC inhibits the opening of the mitochondrial pore and provides protection from injury due to ischemia reperfusion.[19] In addition, there have been several studies showing a role for VDAC in neuronal cell function during ischemia.[20,21] Our study for the first time suggests a correlation between improved cardiac function and a decrease in VDAC 2. Given the role of VDAC in apoptosis, further research is needed to see if VDAC inhibition can prevent adverse cardiac remodeling and if different VDAC isoforms have overlapping or distinct functions.

Our results showed that GPX 1 had a significant exercise-induced expression post-MI. Several groups have shown that exercise increases GPX activity in the heart.[22-24] We know of no previous studies that have measured GPX 1 levels in the post-MI setting.

Ardanaz and colleagues have recently shown that GPX null mice have accelerated cardiac hypertrophy and dysfunction following 7 days of angiotensin II infusion with no effect on blood pressure, indicating a role for protective GPX in cardiac remodeling.[25] Based on this report and our findings, future studies are warranted to determine the functional consequence of exercise on GPX 1 activity post-MI.

Manganese superoxide dismutase (MnSOD) protein increases in response to exercise post-MI. This finding is important because it has been documented that oxidative stress is one cause of adverse LV remodeling post-MI.[26] Given this link, there is ongoing research on possible anti-oxidant therapies to target this adverse outcome of the post-MI heart.[27] There has already been a significant amount of research done on the effect exercise has on increasing antioxidant activity in myocardial cells, with the strongest evidence on MnSOD. [7,28] Yamashita and colleagues demonstrated that inhibition of MnSOD activity blocks the cardioprotective effects of exercise and abolishes the exercise-mediated decrease in infarct size, indicating that a major effect of exercise is to increase MnSOD activity.[29] Alternatively, over-expression of MnSOD in mice results in a markedly reduced infarct size. [29] A later study by Hamilton et al showed that exercise-induced MnSOD prevented cardiac arrhythmias induced by myocardial ischemia and reperfusion.[30] Our results agree with previous literature that increased expression of the MnSOD protein is one of the pathways responsible for the cardioprotective benefits of exercise in the post-MI heart. Because MnSOD is prominent in both skeletal and cardiac muscle biology,[31-33] increased MnSOD levels is likely a major effect in our study. MnSOD levels were also elevated with cardiac aging, indicating that MnSOD may be a common LV mechanism to respond to stress. [17]

One limitation of this study is that the use of 11 cm IPG strips with a focus of 3-10 has decreased resolution compared to 18 or 24 cm IPG strips or IPS strips with a smaller focus range. This means that we are likely missing additional candidate markers. Narrowing in on the focus or using tandem approaches such as gel-free labeling techniques will likely expand the list of candidates identified.

In summary, we identified 3 specific proteins in the post-MI LV that are modified in levels by exercise. Altered glutathione peroxidase, voltage dependent anion channel 2, and manganese superoxide dismutase levels in the exercise group indicate that exercise affects apoptosis and oxidative stress pathways in the post-MI myocardium. The post-MI changes observed in VDAC 2, GPX 1, and MnSOD likely reflect a combined remodeling phenotype, rather than being three proteins reflecting distinct patterns of change and together, these three proteins may be useful candidates to indicate adverse remodeling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Experimental Design

Figure 2.

Representative 2-DE gels of the infarct extracts from the MI-sedentary group (left) and MI-exercise group (right). Spot numbers are listed on the MI-Sed representative gel. There were 435 spots in the MI-Sed representative gel and 453 spots in the MI-Ex representative gel.

Figure 3. Immunoblotting confirmed A) increased glutathione peroxidase, B) decreased voltage-dependent anion-selective channel-2, and C) increased manganese superoxide dismutase in exercise treated rat infarcts. + positive control is a tumor sample homogenate.

Table 1**Echocardiography and Necropsy Results**

	Sham-Sed	Sham-Ex	MI-Sed	MI-Ex
Necropsy				
Heart Rate (bpm)	326±23	331±6	328±8	295±9
Body Weight (g)	445±21	377±10 [*]	424±6	367±12 [†]
Heart Weight to Body Weight (g/g)	2.95±0.15	3.32±0.06	3.99±0.23 [*]	4.57±0.11 ^{*†}
Infarct Size (%)	N/A	N/A	43±2	45±3
Echocardiography (normalized to body weight)				
Anterior Wall Thickness (Systole; mm/g)	0.63±0.03	0.76±0.04 [*]	0.11±0.01 [*]	0.13±0.00 [*]
End Diastolic Volume (ml/g)	1119±148	998±148	3697±116 [*]	4635±126 ^{*†}
End Systolic Volume (ml/g)	184±40	187±7	2423±67 [*]	2761±239 [*]
Stroke Volume (ml/g)	414±46	306±18	541±33	694±75 ^{*†}
Cardiac Output (ml/min/g)	133±15	101±7	177±10	207±28 [*]
Ejection Fraction (%)	84±2	81±1	34±1 [*]	41±4 ^{*†}

^{*} p<0.05 vs Sham-Sed;

^{*} p<0.05 vs respective sham;

[†] p<0.05 vs MI-Sed; n=3-7 each group

Table 2

Fifty-two proteins were identified in the twenty-two spots differentially expressed in the infarct region of sedentary and exercise-treated rats.

Spot No.	MI-Sed Normalized Volume ^a (n=10)	MI-Ex Normalized Volume (n=12)	Protein name	Accession No.
3	621±105	362±37	Actin	gi 7140539
			Long-chain acyl-CoA dehydrogenase	gi 726095
6	1343±61	2169±143	Actin	gi 114794125
19	111±16	162±20	Aldolase A	gi 202837
			Glyceraldehyde 3-phosphate dehydrogenase 1	gi 16304127
			Enoyl coenzyme A hydratase 1, peroxisomal	gi 12018256
25	770±53	1110±71	Cytochrome c oxidase subunit II	gi 26983979
38	756±93	549±43	Mitochondrial aconitase	gi 10637996
39	581±61	423±36	Mitochondrial aconitase	gi 10637996
41	167±19	122±9	Malate dehydrogenase, mitochondrial	gi 38648863
49	1426±100	1103±94	Glyceraldehyde-3-phosphate dehydrogenase	gi 62662278
52	473±32	367±30	Mitochondrial aconitase	gi 10637996
55	6252±403	7920±501	Kininogen	gi 57526868
			Lumican	gi 13591983
56	1515±99	1200±88	Voltage dependent anion channel <i>b2</i>	gi 4105605
58	225±11	180±15	Isocitrate dehydrogenase 2	gi 73587323
			Citrate synthase	gi 18543177
59	768±29	619±30	Glutathione peroxidase <i>b1</i>	gi 1586514
			Manganese superoxide dismutase <i>b1</i>	gi 8394331
			Glutathione S-transferase, pi 2	gi 34849843
			Blvrb protein	gi 34855391
60	1474±55	1191±79	Dihydrolipoamide dehydrogenase	gi 38303871
			Chain A, F1-ATPase	gi 6729934
			Methylmalonate semialdehyde dehydrogenase	gi 13591997
			Aldehyde dehydrogenase 2	gi 38541105
			Sorting and assembly machinery component 50 homolog	gi 14043738
			Albumin	gi 19705431
			3-oxoacid CoA transferase 1	gi 62642974
61	472±27	383±28	Mitochondrial aconitase	gi 10637996
62	499±20	406±36	Creatine kinase <i>b1</i>	gi 38259206
63	700±43	571±40	Guanine deaminase	gi 7533042
			Ubiquinol-cytochrome c reductase core protein I	gi 51948476
			NAD(P) transhydrogenase, beta subunit	gi 71548983

Spot No.	MI-Sed Normalized Volume ^a (n=10)	MI-Ex Normalized Volume (n=12)	Protein name	Accession No.
			Dihydrolipoamide succinyltransferase	gi 71489587
			Desmin	gi 55616124
68	385±20	331±14	Protein disulfide-isomerase A3 precursor	gi 1352384
			Pyruvate kinase, muscle	gi 16757994
			Albumin	gi 19705431
			Chaperonin subunit 6a (zeta)	gi 76253725
			EH-domain containing 1	gi 7106303
			Carboxylesterase 3	gi 31542380
			Catalase	gi 6978607
			Propionyl Coenzyme A carboxylase, beta polypeptide	gi 8393913
			ABC transporter ATP-binding protein	gi 89067622
			Ab2-417	gi 33086606
			Aconitase	gi 3600098
			Pgm1 protein	gi 118764083
			WD repeat protein 1	gi 62078997
			Aldehyde dehydrogenase 4 family, member A1	gi 109475727
69	5342±279	6180±267	14-3-3 protein <i>b1</i>	gi 1526539
			Apolipoprotein B editing complex 2	gi 6753098
70	1826±91	1604±59	Transgelin <i>b2</i>	gi 13928744

^aValues are shown as mean±SEM; p<0.05 for all

^bExamined further by immunoblotting: ¹the protein was previously associated with exercise in skeletal or cardiac muscle or ²the protein has not been previously described in the post-MI

Table 3

Agreement between results observed by 2-DE gel analysis and immunoblotting

Protein	Spot No.	2-DE		Immunoblotting	
		MW	Change	MW	Change
14-3-3	69	33	↑	28	↔
Creatine kinase, mitochondrial 2	62	59	↓	46	↔
Glutathione peroxidase 1	59	26	↓	22	↑
Manganese Superoxide Dismutase	59	26	↓	25	↑
Transgelin	70	21	↓	22	↔
Voltage dependent anion channel 2	56	42	↓	38	↓