Differential Proteome Profiling Analysis of Murine Skeletal Muscle Following a Repeat Bout of Exercise

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ABSTRACT

Skeletal muscle is a plastic tissue that adapts to exercise in many ways. Differential proteome profiling can be used to obtain a global view of proteins that change expression following a repeat bout of eccentrically-biased exercise, as well as its gender specificity. Exercise-naive male and female mice (C57BL/10ScSnJ 000476 +/+) ran downhill twice one week apart (-15°) on a treadmill at 25 m min^{-1} for 15 min and 800 ug of total biceps brachii extract was electrophoresed on pH 5-8 2-D gels. The resulting spots that changed at least +/- 2-fold and were statistically significantly different ($p<0.05$) relative to the unexercised controls using PDQuest 8.0 were analyzed by LC/MS-MS and identified using BioWorks 3.3.1. Significant proteome remodeling occurs subsequent to the repeat bout. The expression change patterns were followed at 0, 24, 48, 72 and 168 hrs post-repeat bout; significant changes were observed in myofibrillar and cytoskeletal proteins, creatine kinase and many of the glycolytic enzymes. The cellular stress responses were unique to individual heat shock and oxidative stress proteins. More protein spots changed in females (299) than in males (236); the female changes peaked at 24 hr post-exercise, whereas the male changes did not show much variation over the week following the repeat bout.

INTRODUCTION

Skeletal muscle tissue responds to a single bout of exercise with macromolecular adaptations. However, this response is greatly attenuated following a second bout of the same activity. This “repeat bout effect” is not well understood, but the adaptations seem to be gender-specific. A comparison of the muscle proteomes of male and female mice following a single and repeated bout of non-damaging, eccentrically-biased contractions will shed light on the underlying molecular mechanisms of these adaptations.

MATERIALS AND METHODS

Our downhill running protocol provides a non-damaging, moderate, eccentrically-biased exercise. Female and male exercise-naive mice (C57BL, n=3) ran for 15 min at 25 m min^{-1} at a -15° incline, with two bouts spaced one week apart. The biceps brachii were excised at 0, 24, 48, 72 and 168 hours post-exercise and extracted directly into the 2D-PAGE buffer [8 M urea, 50 mM DTT, 4% CHAPS, 0.2% carrier ampholytes 5/8].

800 µg muscle extract was separated by 2D-PAGE (pH 5-8, 10.5-14% SDS gel), stained in Coomassie Brilliant Blue R250, imaged and quantified with PDQuest 8.0 (BioRad). Proteins in spots that changed at least 2-fold and were statistically significant ($p<0.05$) relative to the non-exercised controls were excised from the gels, sequenced by tandem mass spectrometry (LC/MS/MS, LCQ Deca XP MAX), and identified by a bioinformatics analysis of the translated mouse genome (BioWorks 3.3.1, BioRad).

Figure 1: 2-D gel of the male control murine biceps brachii. Some of the spots that changed more than 2-fold with statistical significance in at least one time point relative to the exercise-naive control in either gender are labeled **[Figures 2-4]**. Quantitation of these changes may be found in **Table 2**. Proteins are: 1) actin, alpha 1; 2) desmin; 3) superoxide dismutase 1, soluble; 4) malate dehydrogenase 1, NAD, soluble; 5) creatine kinase, muscle (probable ppCK); 6) creatine kinase, muscle (probable pCK); 7) enolase 3, beta, muscle; 8) creatine kinase, muscle; 9) alpha-B crystallin; 10) nucleoside-diphosphate kinase 2.

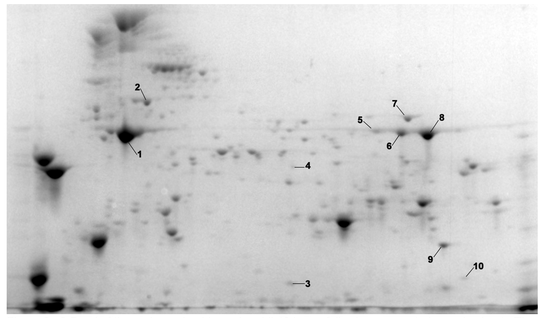

Figure 2: 2-D gel of the male murine biceps brachii 48 hours following a repeated exercise bout. The labeled protein spots and identities are identical to those in **Figure 1**.

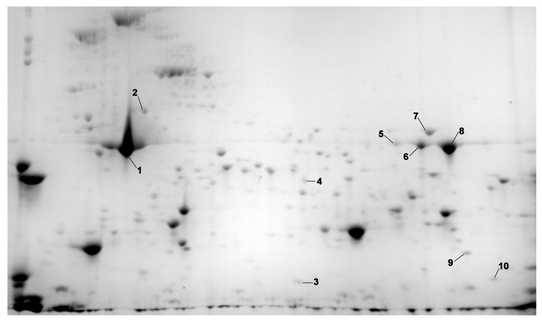

Figure 3: 2-D gel of the female control murine biceps brachii. The labeled protein spots and identities are identical to those in **Figure 1**.

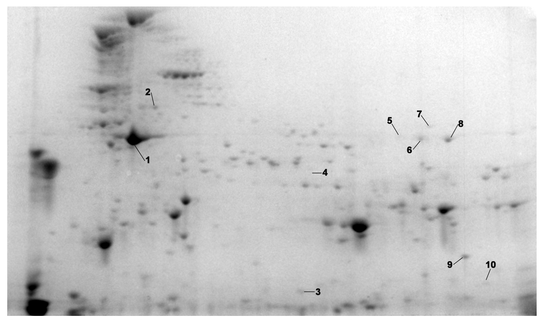

Figure 4: 2-D gel of the female murine biceps brachii 48 hours following a repeated exercise bout. The labeled protein spots and identities are identical to those in **Figure 1**.

		Fold changes with respect to same gender exercise-naive controls						
Protein	**Gender**	**Male Naive**	**1 bout 168hr**	**2 bouts 0hr**	**2 bouts 24hr**	**2 bouts 48hr**	**2 bouts 72hr**	**2 bouts 168hr**
Actin, alpha 1, skeletal muscle	Male		2.6	2.6	2.0	1.5	3.4	2.3
	Female	1.7	-1.3	-1.9	-1.2	-1.0	-1.4	-1.5
Creatine kinase, muscle	Male		-1.4	-1.4	1.1	-2.0	-1.3	-2.1
	Female	-1.3	-1.3	-7.7	-7.1	-5.6	-6.7	-4.5
Creatine kinase, muscle (probable pCK)	Male		1.2	-1.3	1.0	-1.1	1.4	1.4
	Female	1.1	-2.3	-7.1	-4.0	-3.0	-6.3	-2.4
Creatine kinase, muscle (probable ppCK)	Male		1.3	-1.1	1.5	1.3	1.6	2.1
	Female	-1.2	-2.0	-3.8	-3.4	-2.9	-2.1	-1.6
Crystallin, alpha B	Male		3.2	3.0	7.3	5.1	2.2	3.9
	Female	1.6	2.1	1.3	4.1	3.2	2.9	2.6
Desmin	Male		1.7	2.0	2.6	2.0	2.5	2.0
	Female	-1.7	1.6	-1.5	1.1	1.2	1.2	1.1
Enolase 3, beta muscle	Male		-1.1	-1.4	1.3	-2.0	1.1	-1.4
	Female	-1.9	-1.4	-8.3	-7.7	-5.6	-3.7	-2.9
Malate dehydrogenase 1, NAD (soluble)	Male		-1.2	-1.1	-1.2	-2.8	1.2	1.0
	Female	1.3	-2.4	-5.3	-4.2	-5.3	-2.8	-2.1
Nucleoside-diphosphate kinase 2	Male		1.5	-1.9	1.9	-1.9	-1.3	-1.0
	Female	-1.1	1.0	-1.8	-1.6	-3.2	-1.8	-1.8
Superoxide dismutase 1, soluble	Male		3.2	3.2	3.4	4.4	2.7	2.6
	Female	1.6	1.2	1.1	1.2	1.3	1.6	1.6

Table 2: Example protein spot intensity changes over a one-week time course in male and female muscle. All time points were compared separately against their same-gender exercise-naive control, and a student’s t-test performed. Female naive samples were also compared with male naive samples. Entries in blue achieved statistical significance ($p<0.05$). These proteins are associated with KOG subgroups Z, O, C, G, F, and P **[Table 1].**

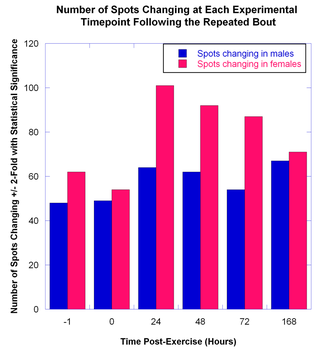

Figure 5: A comparison of the magnitude of response in males and females, measured as the number of total spots changing in a single group when compared with the exercise-naive control of the same gender.

KOG Main Group	KOG Subgroup	Proteins Identified in Sample Gels	M	F
Information Storage and Processing	[K] Transcription	Transformation related protein 63 (p63)	•	
Cellular Processes and Signaling	[D] Cell cycle control, cell division, chromosome partitioning	Internexin neuronal intermediate filament protein, alpha	•	•
	[V] Defense mechanisms	DJ-1 protein	•	
	[T] Signal Transduction Mechanisms	Voltage-dependent anion channel 1	•	
	[Z] Cytoskeleton	Actin, alpha 1	•	•
		Actin, beta cytoplasmic	•	•
		Actin, gamma cytoplasmic 1	•	•
		Actinin, alpha 3	•	•
		Cofilin 2, muscle	•	•
		Desmin	•	•
		Internexin neuronal intermediate filament protein, alpha	•	•
		Microtubule-actin crosslinking factor 1	•	•
		Myosin, heavy polypeptide 1, adult	•	•
		Myosin, heavy polypeptide 2	•	•
		Myosin, heavy polypeptide 3, embryonic	•	•
		Myosin, heavy polypeptide 4	•	•
		Similar to myosin, heavy polypeptide 8, perinatal	•	•
		Myosin, heavy polypeptide 13	•	•
		Myosin light chain, phosphorylatable, fast skeletal muscle	•	•
		Myosin, light polypeptide 1	•	•
		Myosin, light polypeptide 3		•
		Tropomyosin 1, alpha	•	•
		Tropomyosin 2, beta		•
		Troponin T3, skeletal, fast	•	•
		Tubulin, beta	•	
		Vimentin	•	•
	[U] Intracellular trafficking, secretion, and vesicular transport	Internexin neuronal intermediate filament protein, alpha	•	•
	[O] Posttranslational modification, protein turnover, chaperones	Crystallin, alpha B	•	•
		Heat shock 70kD protein 5, glucose-regulated protein (GRP78)		•
		Heat shock protein 1 (HSP25)		•
		Heat shock protein 1(ppHSP25)		•
		Heat shock protein 1, chaperonin (HSP60)	•	•
		Heat shock protein 1A (HSP70)	•	
		Heat shock protein 8 (HSP22)	•	
		Peroxiredoxin 2	•	
		Prohibitin		•
		Proteasome (prosome, macropain) subunit, beta type 6	•	
	[W] Extracellular Structures	Lectin, galactose binding, soluble 1	•	
Metabolism	[C] Energy production and conversion	Aconitase	•	
		Aconitase 2, mitochondrial		•
		ATP synthase, H+ transporting mitochontrial F1 complex, alpha subunit, isoform 1	•	•
		ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	•	•
		Citrate synthase		•
		Creatine kinase, mitochondrial 2		•
		Creatine kinase, muscle (CK)	•	•
		Creatine kinase, muscle (pCK)		•
		Creatine kinase, muscle (ppCK)	•	•
		Dihydrolipoamide dehydrogenase	•	
		Dihydrolipoamide S-acetyltransferase	•	
		Dihydrolipoamide S-succinyltransferase		•
		Isocitrate dehydrogenase 2, mitochondrial		•
		Isocitrate dehydrogenase 3, beta subunit	•	•
		Lactate dehydrogenase 1, A chain		•
		Lactate dehydrogenase 2, B chain	•	
		Malate dehydrogenase 1, NAD (soluble)	•	•
		Malate dehydrogenase 2, NAD (mitochondrial)	•	•
		Myoglobin	•	•
		Myozenin	•	
		Pyruvate dehydrogenase E1 alpha 1	•	
		Pyruvate dehydrogenase (lipoamide) beta		•
		Succinate-Coenzyme A ligase, ADP-forming, beta subunit		•
		Ubiquinol-cytochrome c reductase core protein 1	•	•
		Ubiquinol-cytochrome c reductase core protein 2		•
	[G] Carbohydrate transport and metabolism	Aldolase 1A	•	•
		Aldolase 3C		•
		Enolase 3, beta	•	•
		Glyceraldehyde-3-phosphate dehydrogenase	•	•
		Pyruvate kinase 3	•	•
		Pyruvate kinase 4		•
		Triosephosphate isomerase 1	•	•
	[E] Amino acid transport and metabolism	Aconitase	•	
		Aconitase 2, mitochondrial		•
		Isocitrate dehydrogenase 2, mitochondrial		•
		Isocitrate dehydrogenase 3, beta subunit	•	•
		Isovaleryl coenzyme A dehydrogenase		
	[F] Nucleotide transport and metabolism	Adenylate kinase 1		•
		Nucleoside-diphosphate kinase 1		•
		Nucleoside-diphosphate kinase 2	•	•
	[I] Lipid transport and metabolism	Acetyl-Coenzyme A acyltransferase 2		•
		Fatty acid binding protein 3	•	
		Isovaleryl coenzyme A dehydrogenase	•	
	[P] Inorganic ion transport and metabolism	Superoxide dismutase 1, soluble	•	
		Voltage-dependent anion channel 1	•	
Poorly Characterized	[R] General Function Prediction Only	Carbonic anhydrase		•
	[S] Function unknown	Hypothetical protein 4732456N10	•	•
		Hypothetical protein LOC238880	•	

Table 1: Unique protein identifications of spots changing +/- 2-fold with statistical significance in male (M) and female (F) biceps brachii following a repeated bout of downhill running exercise. Proteins are classified according to the NCBI KOG database, with all 4 main groups and 16 of 24 subgroups represented.

RESULTS

A. 2D-PAGE mouse biceps brachii sample gels display 600-800 protein spots with pI 5 to 8 and Mr 14 to 250 kDa **[Figures 1-4]**.

B. Females showed a greater response than males (236 total spots met excision criteria in males; 299 in females) **[Figure 5]**. The timecourse of the response is also different, with a distinct female peak at 24 hours post-exercise and less variation in males over the week.

C. 56 unique proteins changed significantly more than 2-fold ($p<0.05$) in the male proteome in at least one time point, and 70 in the female proteome. These proteins classified into 3 main KOG groups, and 13 subgroups **[Table 1]**.

D. Quantitation of protein expression changes shows specific time courses over the week following a repeated bout of exercise **[Table 2]**.

CONCLUSIONS

1. The dynamic nature of the mouse biceps brachii proteome has been demonstrated following two non-damaging, eccentrically-biased exercise bouts: 236 male and 299 female protein spots changed +/- 2-fold with statistical significance ($p < 0.05$).

2. The proteome response to exercise is gender-specific, with males and females exhibiting both different magnitudes and different time courses of response.

3. In male mice, 56 unique proteins have been identified, showing a targeted response among proteins functioning in the sarcomere and cytoskeleton, metabolism and the stress response. In female mice, the 70 unique proteins identified show similar targeting.

4. The presence of non-adult myosin heavy polypeptide isoforms in both genders, evidence of sarcomere protein fragmentation, and actin concentration increase in males indicate sarcomere remodeling occurring over the week following the repeated bout.

ACKNOWLEDGEMENTS

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