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Proteomic analysis of rat soleus muscle undergoing hindlimb suspension-induced atrophy and reweighting hypertrophy

A proteomic analysis was performed comparing normal rat soleus muscle to soleus muscle that had undergone either 0.5, 1, 2, 4, 7, 10 and 14 days of hindlimb suspension-induced atrophy or hindlimb suspension-induced atrophied soleus muscle that had undergone 1 hour, 8 hour, 1 day, 2 day, 4 day and 7 days of reweighting-induced hypertrophy. Muscle mass measurements demonstrated continual loss of soleus mass occurred throughout the 21 days of hindlimb suspension; following reweighting, atrophied soleus muscle mass increased dramatically between 8 hours and 1 day post reweighting. Proteomic analysis of normal and atrophied soleus muscle demonstrated statistically significant changes in the relative levels of 29 soleus proteins. Reweighting following atrophy demonstrated statistically significant changes in the relative levels of 15 soleus proteins. Protein identification using mass spectrometry was attempted for all differentially regulated proteins from both atrophied and hypertrophied soleus muscle. Five differentially regulated proteins from the hindlimb suspended atrophied soleus muscle were identified while five proteins were identified in the reweightinginduced hypertrophied soleus muscles. The identified proteins could be generally grouped together as metabolic proteins, chaperone proteins and contractile apparatus proteins. Together these data demonstrate that coordinated temporally regulated changes in the skeletal muscle proteome occur during disuse-induced soleus muscle atrophy and reweighting hypertrophy.

Keywords: Atrophy / Hypertrophy / Skeletal muscle

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1 Introduction

Skeletal muscle atrophy is a process by which skeletal muscle, in response to a variety of stimuli, selectively lose proteins; hypertrophy is in essence a reverse of this process [1–5]. Skeletal muscle atrophy can be induced by diverse stimuli such as disuse, denervation, sepsis, and starvation while hypertrophy is induced by increase muscle utilization [1-5]. While each atrophy-inducing stimuli has its own unique initiation signal, it is generally believed that a common mechanism of protein loss occurs independent of the inducing stimuli. The selective loss of a subset of skeletal muscle proteins including contractile proteins results in muscles with smaller myofibers but no loss of myofiber numbers; in contrast hypertrophy results in a selective increase in skeletal muscle proteins and myofiber size without an increase in myofiber number [1-5]. It is generally thought that atrophy and hypertrophy are

Correspondence: Dr. Robert J. Isfort, Research Division, Procter & Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason-Montgomery Road, Mason, OH 45040-9317, USA E-mail: isfortrj@pg.com Fax: +1-513-622-1195 physiological mechanisms that function to meet the work demand placed on the muscle and to store and release amino acids during times of physiological need.

Skeletal muscle atrophy occurs by both decreasing protein synthesis and increasing protein degradation; in contrast skeletal muscle hypertrophy occurs by decreasing protein degradation and increasing protein synthesis [6-9]. Mechanistically, this occurs by altering both gene transcription and mRNA translation on the protein synthesis side and modulating proteolysis including activating the calpain, lysosomal and ubiquitin-mediated proteolytic systems on the protein degradation side [6-9]. While contractile proteins appear to be selectively lost or gained during skeletal muscle atrophy or hypertrophy, the available literature indicates that additional protein alterations occur in noncontractile proteins [1]. Thus the process of skeletal muscle atrophy and hypertrophy is probably more complex than has been previously recognized. In order to gain a better understanding of the protein changes that occur during hindlimb suspension-induced skeletal muscle atrophy and reweighting-induced skeletal muscle hypertrophy, a proteomic analysis was performed on soleus muscle undergoing either atrophy or hypertrophy. The results of that analysis are reported here.

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2 Materials and methods

2.1 Hindlimb suspension and reweighting

Rat hindlimb suspension was performed as has been described previously [10]. At the indicated times, animals were removed from the suspension apparatus, euthanized by CO_2 asphyxiation followed by cervical dislocation, the right soleus muscle was dissected from the hindlimb, cleaned of tendons and connective tissue, weighed and snap frozen in cryogenic vials by immersion in liquid nitrogen. Muscle were stored at $-80^{\circ}C$ until processed. For the reweighting analysis, 21 d hindlimb suspended rats were removed from the suspension apparatus and allowed to move freely in the cage. At the indicated times right soleus muscles were collected as described above.

2.2 Sample preparation

The soleus muscle was prepared for 2-DE as follows. Frozen soleus muscles were crushed to a fine powder in liquid nitrogen using a mortar and pestle, 0.1 g of tissue was solubilized using homogenization in 0.4 mL of solubilization buffer (9 mmu urea, 2% CHAPS, 0.5% DTT, 2% pH 8.0–10.5 Pharmalytes, Amersham Biosciences, Uppsala, Sweden), shaken for approximately 30 min, centrifuged for 30 min, and the supernatant removed and aliquotted for analysis.

2.3 2-DE

Sample proteins were resolved by 2-DE using the 20 \times 25 cm ISO-DALT 2-D gel system (Large Scale Biology, Germantown, MB, USA) essentially as described previously [11–13]. All first dimension IEF gels were prepared using the same single standardized batch of carrier ampholytes (Resolyte 4-8, BDH, Poole, UK) by combining urea, carrier ampholytes and acrylamide solution to cast the tube gels; casting the tube gels and allowing to polymerize for at least 1 h; loading the tube gel into the IEF apparatus and adding buffer; and prefocusing the gel for 1 h at 200 V. Eight microliters of solubilized muscle protein were applied to each gel and the gels were run for 25050 Vhr using a progressively increasing voltage protocol implemented by a programmable high voltage power supply. An Angelique computer controlled gradient casting system (Large Scale Biology) was used to prepare second dimension SDS gradient slab gels in which the top 5% of the gel was 11%T acrylamide and the lower 95% of the gel varies linearly from 11-18%T. The first dimensional IEF tube gels were loaded directly onto the slab gels without equilibration and held in place by agarose. Second dimension slab gels were run in groups of 20 in thermal-regulated DALT tanks with buffer circulation. Following SDS electrophoresis, slab gels were

stained for protein using a colloidal Coomassie Blue G-250 procedure involving fixation in 50% ethanol/2% phosphoric acid overnight, three 30 min washes in cold deionized water, transfer to 34% methanol/17% ammonium sulfate/2% phosphoric acid for 1 h followed by addition of 1 g/1.5 L of powdered Coomassie Blue G-250 stain and staining for 4 d. Samples were analyzed at least twice: if adequate resolution was not achieved on one of the gels, additional gel runs were performed.

2.4 Quantitative computer analysis

Each stained slab gel was digitized in red light at 133 micron resolution using an Eikonix 1412 scanner (Bedford, MA, USA). Each 2-D gel was processed using the Kepler software system (Large Scale Biology) to yield a spotlist giving position, shape and density information for each detected spot. This procedure makes use of digital filtering, mathematical morphology techniques and digital masking to remove background and uses full two-dimensional least squares optimization to refine the parameters of a 2-D Gaussian shape for each spot. Each 2-D pattern was matched to an appropriate soleus "master" 2-D pattern. In the matching, a series of about 50 proteins was matched by an experienced operator working with a montage of all the 2-D patterns in the experiment. Subsequently, an automatic program matched additional spots to the master pattern using as a basis the manual landmark data entered by the operator. After the automatic matching, the operator inspected matching for errors. The groups of gels making up an experiment were scaled together by a linear procedure based on a selected set of spots. These spots were selected by a procedure that chooses spots that have been matched in at least 80% of the gels. All gels in the experiment were then scaled together by setting the summed abundance of the selected spots equal to a constant. A student's t-test was used to evaluate the level of significance of any quantitative change in the level of analyzed proteins between control and each time-point after the induction of atrophy or hypertrophy. A response was considered significant if the difference between control and any time-point achieved the p<0.01 significance level (*n* = 3 *per* protein spot *per* time-point).

2.5 Protein identification

Protein spots of interest were excised from the Coomassie Blue stained gels and in-gel tryptic digestion performed as described previously [16, 17]. Briefly, the spots were washed with 100 mM NH₄HCO₃/50% CH₃CN several times followed by reduction and alkylation with DTT and iodoacetamide respectively. After washing with 100 mM NH₄HCO₃/50% CH₃CN, 0.15–0.2 μ g of porcine trypsin in 100 mM NH₄HCO₃ was added to the gel pieces followed by an overnight incubation at 30°C. Protein identification was performed using MALDI-TOF MS as follows. Mass spectrometry of the tryptic fragments was performed by mixing the peptides from in-gel trypsin digestion with an equal volume of saturated *a*-cyano-4-hydroxycinnamic acid matrix dissolved in 50% CH₃CN/03% TFA, then spotted onto a MALDI-TOF MS target plate. Peptide spectra were collected on a PerSeptive Biosystems (Framingham, MA, USA), Voyager DE-STR MALDI-TOF mass spectrometer in the positive ion, reflector mode with delayed ion extraction using the following conditions: nitrogen laser at 337 nm, accelerating voltage at 20 kV, grid voltage at 73%, ion delay at 100 ns and a mass range of 800-3800 Da. The mono-isotopic masses were calibrated to internal, autodigestion peptides from porcine trypsin at 842.5100 and 2211.1046 such that the unknown peptide masses were accurate to within 25 ppm. Protein identification was facilitated using the MS-Fit module of the ProteinProspector (V3.2.1) program, supplied by PerSeptive Biosystems. The accurate tryptic, monoisotopic peptide masses were searched against the entire NCBI database to match with tryptic peptide masses from known proteins and/or predicted protein from DNA sequences. All spectra were internally calibrated using two spiked peptides and databases searched with a mass tolerance of 25 ppm.

In the event that the protein identification was ambiguous after the MALDI-TOF step, the remaining peptide sample was analyzed by capillary LC-ESI-MS/MS with an LC Packings (Amsterdam, The Netherlands) Ultimate Capillary LC system equipped with a FAMOS micro-autosampler and a 5 cm \times 300 μ m id PepMap C18 column coupled to a Finnigan (San Jose, CA, USA) LCQ^{Deca} ion-trap mass

spectrometer. A gradient was developed over 30 min at 4 μ L/min from 2–49% acetonitrile containing 0.1% formic acid to elute the peptides from the column directly into the mass spectrometer. Data were collected and analyzed using Finnigan Xcalibur V1.2 software in data-dependent scan mode such that any peptide signal over 1 × 10⁵ intensity triggered the automated acquisition of an MS/MS fragmentation spectrum for that peptide. The collective MS/MS spectra for each capillary LC-MS/MS run were searched against the NCBI database using Mascot Daemon (V1.7.1) as a client attached to an in-house Mascot search protocol server (http://www.matrixscience.com).

3 Results

3.1 Soleus muscle mass changes during hindlimb suspension and reweighting following hindlimb suspension

Atrophy of the soleus muscle was induced by hindlimb suspension while hypertrophy of the soleus was induced by reweighting the hindlegs after 21 d of hindlimb suspension. Soleus muscles were removed from the right leg of three control rats as well as the right legs of hindlimb suspended rats (3 *per* time point) at 0.5 d, 1 d, 2 d, 4 d, 7 d, 10 d and 14 d post hindlimb suspension; soleus muscles were removed from the right leg of three 21 d hindlimb suspended rats (3 *per* time point) at 1 h, 8 h, 1 d, 2 d, 4 d and 7 d post reweighting. All removed soleus muscles were weighed and then prepared for 2-D gel analysis. The changes in soleus muscle mass following hindlimb suspension are presented in Fig. 1A. As can be seen, the soleus muscle



Figure 1. Analysis of rat soleus muscle mass changes following either hindlimb suspension or reweighting after hindlimb suspension. For hindlimb suspension, rats were suspended by the tail and the soleus muscle was removed at either 0, 0.5, 1, 2, 4, 7, 10 and 14 d post suspension and weighed (n = 3 for each time-point). For reweighting, the rats were suspended by the tail for 21 d (day 0) after which they were unsuspended and the soleus muscle was removed at either 0, 1 h, 8 h, 1, 2, 4, and 7 d post reweighting and weighed (n = 3 for each time-point). A. Hindlimb suspension soleus weight data; B, reweighting following hindlimb suspension soleus weight data.



Figure 2. Alterations in the relative levels of soleus muscle proteins following hindlimb suspension. Soleus muscle proteins were prepared and separated by 2-DE as described in Section 2.3. A. Master map of soleus muscle proteins (clear spots) showing proteins whose levels changed following hindlimb suspension (black spots); B, graphical representation of the 29 soleus proteins with relative level changes during hindlimb suspension. Each tic separates a group of three protein spots (the same protein), each from a different soleus muscle from three different animals. Time-points following hindlimb suspension). Each panel, which represents the integrated Coomassie Blue absorbance measurement for one protein, is the relative measure of the normalized abundance of that protein from the different experimental groups (y-axis) *versus* experimental group (x-axis).

lost approximately 50% of its mass by 14 d of hindlimb suspension with mass loss occurring throughout the period of hindlimb suspension. In contrast, soleus muscle mass increased dramatically between 1 and 2 d post reweighting with the muscle achieving its preatrophy levels at 2 d post reweighting as demonstrated in Fig. 1B.

3.2 Changes in soleus muscle protein levels following hindlimb suspension and reweighting after hindlimb suspension

Proteomic analysis of normal and hindlimb suspended soleus muscle proteins was performed using samples isolated at 0, 0.5, 1, 2, 4, 7, 10 and 14 d post unweighting. The normal soleus protein pattern was used to create a soleus muscle proteome master map that was subsequently used for landmarking and identifying proteins whose levels change during hindlimb suspension-induced atrophy. As can be seen in Fig. 2A, 29 proteins whose levels changed at the p<0.01 level of significance in any group were identified (these proteins are denoted by their coordinate numbers and dark shading relative to all pro-

teins resolved, represented by light shading). These proteins cover a broad range of molecular weights, p/s and abundance levels (the size of the spot indicates relative abundance). Figure 2B demonstrates the change in relative abundance of the 29 proteins at varying times following hindlimb suspension. Each tic separates a group of three protein spots (the same protein) with each protein spot coming from a different soleus muscle with the three different soleus muscles coming from three different animals. Several different patterns of change are observed, including a gradual increase in relative protein abundance occurring during the entire period of hindlimb suspension (a total of 7 spots - spots 137, 140, 154, 189, 457, 459, 637), a gradual decrease in relative protein abundance occurring during the entire period of hindlimb suspension (a total of 2 spots - spots 255 and 347), a transient increase in relative protein abundance occurring at the 0.5 d time-point followed by a decrease in relative abundance (a total of 2 spots - spots 196 and 556), a decrease in relative protein abundance occurring by the 0.5 d timepoint with the decrease maintained throughout the period of hindlimb suspension (a total of 4 spots - spots 132, 151, 156, 160), and other changes in relative protein abundance (the remaining 14 spots).



Figure 3. Alterations in the relative levels of soleus muscle proteins following reweighting. Soleus muscle proteins were prepared and separated by 2-DE as described in Section 2.3. A. Master map of soleus muscle proteins (clear spots) showing proteins whose levels changed following reweighting (black spots). B, graphical representation of the 15 soleus proteins with relative level changes during reweighting. Each tic separates a group of three protein spots (the same protein), each from a different soleus muscle from three different animals. Time-points following hindlimb suspension are identified by each tic mark (from left to right – 0, 0.5, 1, 2, 4, 7, 10 and 14 d post hindlimb suspension). Each panel, which represents the integrated Coomassie Blue absorbance measurement for one protein, is the relative measure of the normalized abundance of that protein from the different experimental groups (y-axis) *versus* experimental group (x-axis).

Proteomic analysis of 21 d hindlimb suspended and reweighted following 21 d of hindlimb suspension soleus muscle proteins was performed using samples isolated after 21 d of hindlimb suspension and 1 h, 8 h, 1 d, 2 d, 4 d and 7 d post reweighting after 21 d of hindlimb suspension. As demonstrated in Fig. 3A, 15 proteins whose levels changed at the p<0.01 level of significance in any group were identified (these proteins are denoted by their coordinate numbers and dark shading relative to all proteins resolved, represented by light shading). These 15 proteins cover a broad range of molecular weights, pls and abundance levels (the size of the spot indicates relative abundance). Figure 3B demonstrates the relative change in the level of abundance of the 15 proteins at varying times following reweighting. As before, each tic separates a group of three protein spots (the same protein) with each protein spot coming from a different soleus muscle and with the three different soleus muscles coming from three different animals. As before, several different patterns of change are observed including an increase in relative protein abundance occurring between 8 h and 1 d post reweighting (a total of 5 spots - spots 201, 222, 255, 333, 347), a decrease in relative protein abundance occurring between 8 h and 1 d post reweighting (a total of 3 spots - spots 148, 196, 515), a decrease

in relative protein abundance occurring between 1 h and 8 h post reweighting (a total of 4 spots – spots 168, 322, 392, 1988), a gradual decrease in relative protein abundance occurring throughout the period of reweighting (a total of 2 spots – spots 212 and 409), and other changes in relative abundance (1 spot). The decreases or increases observed are compared as the average change observed, and are typically observed in two or more samples/group.

3.3 Protein identification

Protein identification using MALDI-TOF MS and LC-MS/ MS was attempted for all proteins demonstrating statistically significant changes in relative protein levels during hindlimb suspension induced atrophy and reweighting induced hypertrophy. Table 1 provides the protein identification for five soleus proteins whose levels of expression were altered during hindlimb suspension-induced atrophy and five soleus proteins whose levels of expression were altered during reweighting following hindlimb suspension-induced atrophy. The five identified proteins that were altered during hindlimb suspension were β -enolase (protein S196), H+ transporting ATP synthase beta chain

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Table 1	. Protein	identification c	of soleus	proteins	demonstratin	g relative	expression	level	changes	during	either	hindlimb
	suspen	sion or reweigh	ting									

Protein spot number	Protein identification	MOWSE or SEQUEST ^{a)} Xcorr/dCN score	Peptides matched	Predicted <i>M</i> _r /p/	Hindlimb suspension or reweighting
S132	Troponin T	422	VDFDDIHR EEEEAKKR MRKEEEEAK KKEEEELIALKDR	31.1 kDa/5.7	Hindlimb suspension
S148	Lactate dehydrogenase B	256	IVVVTAGR FIIPQIVK MVVDSAYIVIK SLADELALVDVLEDK LIAPVADDETAVPNNK	36.6 kDa/5.7	Reweighting
S156	Troponin T	1080	VDFDDIHR VDFDDIHRK VLSNMGAHFGGYLVK KKEEEELIALKDR IPEGERVDFDDIHR KKPLNIDYMGEDQLR EEERPKPSRPVVPPLIPPK	31.1 kDa/5.7	Hindlimb suspenion
S189	H+ transporting ATP synthase beta chain	3.94/0.22 ^{a)} 3.47/0.37 ^{a)} 3.24/0.47 ^{a)} 2.81/0.49 ^{a)} 2.68/0.4 ^{a)} 1.79/0.28 ^{a)}	IMNVIGEPIDER VALVYGQMNEPPGAR VVDLLAPYAK TIAMDGTEGLVR IPVGPETLGR IPSAVGYQPTLATDMGTMQER	51.2 kDa/4.9	Hindlimb suspension
S196	β-enolase	4.15/0.62 ^{a)} 3.67/0.53 ^{a)} 3.41/0.06 ^{a)} 2.1/0.43 ^{a)} 1.68/0.01 ^{a)} 1.38/0.02 ^{a)}	AAVPSGASTGIYEALELR VVIGMDVAASEFYR GNPTVEVDLHTAK TIGPALIEK YKENMQK YDLDFK	47.0 kDa/7.6	Hindlimb suspension and reweighting
S204	Cardiac myosin light chain 2	904	DTAFAALGR DGFIDKNDLR EAFTIMDQNR EMLTTQAER	18.9 kDa/4.9	Hindlimb suspension
S222	Cardiac myosin light chain 2	1350	DTAFAALGR NEEIDEMIK DGFIDKNDLR EAFTIMDQNR	18.9 kDa/4.9	Reweighting
S333	P20	867	VPVQPSWLR VVGDHVEVHAR ASAPLPGFSTPGR RASAPLPGFSTPGR HEERPDEHGFIAR	17.5 kDa/6.1	Reweighting
S515	Carbonic anhydrase III	1330	GGPLSGPYR YNTFGEALK VVFDDTFDR YAAELHLVHWNPK EPMTVSSDQMAKLR VVFDDTFDRSMLR EKGEFQILLDALDK HDPSLQPWSVSYDPGSAK	29.4 kDa/6.4	Reweighting

(protein S189), troponin T (protein S132), troponin T (protein S156), and cardiac myosin light chain 2 (protein S204); the five identified proteins that were altered during reweighting following hindlimb suspension were carbonic anhydrase III (protein S515), p20 (protein S333), cardiac myosin light chain 2 (protein S222), β -enolase (protein S196), and lactate dehydrogenase B (protein S148). All other proteins from the atrophied and reweighted soleus muscle were either unable to be identified (provided no useful mass spectra) or were novel proteins, *i.e.* proteins which produced useful mass spectra without a match in the protein database. Importantly, our attempts to identify proteins were hampered by an unknown contaminant in the gels that interfered with the mass identification and greatly reduced our sensitivity of detection.

4 Discussion

This paper describes a proteomic analysis of the rat soleus muscle undergoing either hindlimb suspensioninduced atrophy or reweighting-induced hypertrophy. During hindlimb suspension-induced atrophy, we observed statistically significant changes in the relative quantities of 29 proteins; during reweighting-induced hypertrophy, we observed changes in the relative quantities of 15 proteins. Importantly, different classes of protein changes were observed indicating coordinated regulation of protein levels during both hindlimb suspension-induced atrophy and reweighting-induced hypertrophy. Also of significance was the observation that proteins with similar functionality including metabolic, contractile apparatus and chaperone proteins were altered.

4.1 Common protein changes

In this analysis we observed changes in the relative levels of the same protein during hindlimb suspension-induced atrophy and reweighting-induced hypertrophy as follows. (1) Protein S196, which was identified as β -enolase, levels were increased by 12 h following hindlimb suspension, remained elevated during hindlimb suspension, and fell to pre-atrophy levels by 10 d post hindlimb suspension. During reweighting, the relative levels of protein S196 decreased between 1 and 2 d after reweighting. In a previous analysis of denervation-induced atrophy, β-enolase was observed to increase during atrophy [14]. Importantly, the protein spot identified as β -enolase in the denervation-induced atrophy study had the correct molecular weight and pl for rat β -enolase (mass = 46.9 kDa, pl = 7.59); however the protein spot identified as β -enolase in this study was a different spot with a lower molecular mass and a more acidic pl. Thus the spot identified in this study as β -enolase is probably a fragment of β -enolase. (2) Protein S255 levels decreased following hindlimb suspension and increased following reweighting. Interestingly, protein S255 also decreased during denervation-induced atropy with kinetics very similar, if not identical, to that observed during hindlimb suspensioninduced atrophy [14]. Attempts at identifying protein S255 have to date been unsuccessful. (3) Protein S347 levels decreased following hindlimb suspension and increased following reweighting. Attempts at identifying protein S347 have not been successful due to a lack of a match in the protein databases with the peptides generated by MALDI-TOF analysis. Because of the commonality of change of these three proteins during both hindlimb suspension-induced atrophy and reweighting hypertrophy, these three proteins may function as useful markers of the process.

4.2 Classes of altered proteins

The altered identified proteins can be grouped into three functional classes including metabolic enzymes, contractile apparatus proteins and chaperone proteins.

Metabolic proteins which were altered during hindlimb suspension-induced atrophy include β -enolase (S196) and H+ transporting ATP synthase beta chain (S189); the metabolic proteins altered during reweighting-induced hypertrophy include carbonic anhydrase III (S515), β-enolase (S196), and lactate dehydrogenase B (S148). In our previous study on denervation induced atrophy we observed changes in the relative levels of β -enolase (increased), H+ transporting ATP synthase beta chain (increased), and carbonic anhydrase III (increased) [14]. Interestingly, in the current studies we observed similar relative level changes in these proteins independent of the atrophy inducing event (β -enolase increase, H+ transporting ATP synthase beta chain increase) and inverse relative level changes that appear to be dependent on the atrophy inducing event (carbonic anhydrase - increased in denervation-induced atrophy and decreased during reweighting induced hypertrophy). Thus several of these proteins may serve as useful markers of skeletal muscle atrophy and hypertrophy.

Contractile apparatus proteins which were altered during hindlimb suspension-induced atrophy include troponin T (S132 and S156) and cardiac myosin light chain 2 (S204); contractile apparatus proteins which were altered during reweighting-induced hypertrophy include cardiac myosin light chain 2 (S222). This set of protein changes is interesting for several reasons, including the observation that the changes in the relative levels of troponin T were observed in two migrational variants of this protein during

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hindlimb suspension; during denervation-induced atrophy, we observed a decrease in troponin T protein levels at the 0.5 d time-point similar to hindlimb suspension [14]; and troponin T protein levels were observed to decrease during vitamin E deficiency-induced atrophy [15]. Thus troponin T appears to be a good early marker for skeletal muscle atrophy. Cardiac myosin light chain 2 was also observed to decrease during hindlimb suspension-induced atrophy and increase during reweightinginduced hypertrophy. Interestingly, two different proteins were identified as cardiac myosin light chain 2 with spot S204 migrating with a slightly higher molecular weight than spot S222, even though both spots migrated with approximately equivalent p/s. In our previous proteomic analysis of denervation-induced soleus muscle atrophy, we also observed decreased levels of cardiac myosin light chain 2 (S222) [14]. Interestingly, cardiac myosin light chain 2 protein levels (a light chain that is expressed in both slow twitch skeletal muscle and cardiac muscle) were observed to increase during cardiac hypertrophy, indicating that this protein may be a good marker of both slow twitch skeletal muscle and cardiac muscle atrophy and hypertrophy. We are currently investigating the protein migrational differences in both troponin Tand cardiac myosin light chain 2.

The chaperone protein p20 (S333) was altered during reweighting-induced hypertrophy. The protein levels of P20 and related members of the small heat shock protein family have been previously observed by us to change during denervation-induced atrophy [14]; in addition others have also observed changes in the relative levels of P20 during denervation-induced atrophy [16]. Although p20 relative protein levels were not observed to be altered at the p<0.01 level of significance during hindlimb suspension-induced atrophy, a trend of decreased relative P20 protein levels is observed (data not shown), indicating that the P20 protein levels decrease during atrophy in general. Thus P20 may serve as a useful marker of skeletal muscle atrophy.

5 Concluding remarks

What do the alterations in relative protein levels teach us about the process of hindlimb suspension-induced atrophy and reweighting-induced hypertrophy? Based on reciprocity of response by several of the proteins, these results show that in certain regards atrophy and hypertrophy appear to be reciprocal processes. Thus, the identified proteins whose relative expression levels change in opposite directions during atrophy and hypertrophy may be useful markers of atrophy and hypertrophy. In addition, the identification of multiple variants of a protein, including what appear to be proteolytic fragments and migrationally altered proteins, demonstrates the power of proteomics to not only detect guantitative protein changes but also qualitative protein changes, changes that may be important in understanding the process of atrophy and hypertrophy. Finally the observation that metabolic, contractile apparatus and chaperone proteins are altered during atrophy and hypertrophy indicate that these general classes of proteins are selectively altered during atrophy and hypertrophy and thus provide important mechanistic understanding of these physiological processes.

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