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Gene expression profiling in human skeletal muscle during recovery from eccentric exercise

D. J. Mahoney,[1]* A. Safdar,[4]* G. Parise,[2] S. Melov,[3] Minghua Fu,[4] L. MacNeil,[2] J. Kaczor,[4] E. T. Payne,[1] and M. A. Tarnopolsky[4]

Departments of [1]Medical Sciences, [2]Kinesiology, [4]Pediatrics and Medicine, McMaster University, Hamilton, Ontario, Canada; and [3]Buck Institute for Aging Research, Novato, California

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Mahoney DJ, Safdar A, Parise G, Melov S, Fu M, MacNeil L, Kaczor J, Payne ET, Tarnopolsky MA. Gene expression profiling in human skeletal muscle during recovery from eccentric exercise. *Am J Physiol Regul Integr Comp Physiol* 294: R1901–R1910, 2008. First published March 5, 2008; doi:10.1152/ajpregu.00847.2007.—We used cDNA microarrays to screen for differentially expressed genes during recovery from exercise-induced muscle damage in humans. Male subjects ($n = 4$) performed 300 maximal eccentric contractions, and skeletal muscle biopsy samples were analyzed at 3 h and 48 h after exercise. In total, 113 genes increased 3 h postexercise, and 34 decreased. At 48 h postexercise, 59 genes increased and 29 decreased. On the basis of these data, we chose 19 gene changes and conducted secondary analyses using real-time RT-PCR from muscle biopsy samples taken from 11 additional subjects who performed an identical bout of exercise. Real-time RT-PCR analyses confirmed that exercise-induced muscle damage led to a rapid (3 h) increase in sterol response element binding protein 2 (*SREBP-2*), followed by a delayed (48 h) increase in the SREBP-2 gene targets Acyl CoA:cholesterol acyltransferase (*ACAT*)-2 and insulin-induced gene 1 (*insig-1*). The expression of the *IL-1 receptor*, a known regulator of SREBP-2, was also elevated after exercise. Taken together, these expression changes suggest a transcriptional program for increasing cholesterol and lipid synthesis and/or modification. Additionally, damaging exercise induced the expression of *protein kinase H11*, capping protein Z alpha (*capZα*), and modulatory calcineurin-interacting protein 1 (*MCIP1*), as well as cardiac ankryin repeat protein 1 (*CARP1*), *DNAJB2*, *c-myc*, and *junD*, each of which are likely involved in skeletal muscle growth, remodeling, and stress management. In summary, using DNA microarrays and RT-PCR, we have identified novel genes that respond to skeletal muscle damage, which, given the known biological functions, are likely involved in recovery from and/or adaptation to damaging exercise.

mRNA analysis; sterol response element binding protein

SKELETAL MUSCLE IS AN EXTREMELY plastic tissue that has a large capacity to recover from and adapt to damage. The molecular mechanisms responsible for muscle recovery and remodeling after damage are currently being unraveled, and there is accumulating evidence that gene-specific transcriptional activation is involved at some level (2, 8, 11, 12, 22, 43, 62). During recovery from eccentric exercise, a commonly used model of muscle damage in animals and humans, elevations in mRNA encoding myogenic regulatory factors (e.g., MyoD, myogenin), muscle contractile and/or structural proteins (e.g., desmin, vimentin, myosin heavy chain), proteolytic enzymes (e.g., calpain), and putative stress-responsive genes (e.g., uncoupling protein 3) have been observed (8, 22, 43).

To screen for novel genes that respond to muscle damage, various groups have used DNA microarray technology to assess global mRNA expression during recovery from an acute bout of damaging exercise (2, 11, 12, 30, 62). In general, these studies characterized programs of gene expression that represent key events involved in recovery from and adaptation to muscle damage, such as protein synthesis, cellular growth, inflammation, and muscle repair. Moreover, each of these studies identified differently expressed genes that had not been previously reported. For instance, Barash et al. (2) discovered that muscle LIM protein (*MLP*) and muscle ankryin repeat protein 1 and 2 (*MARP1* and *2*, also known as *CARP1* and *2*) were elevated following damaging exercise. Given that these proteins interact with structural proteins in skeletal muscle and may be involved in signal transduction for muscle differentiation (23, 29), these authors speculated that MLP and MARP1/2 may act as mechanical sensors in skeletal muscle that respond to stretch and/or damage. Chen et al. (12) discovered that several gene targets of the stress-responsive protein p53, such as *PC3* and *GADD45*, were elevated following damaging exercise. Subsequent immunoblot analysis revealed elevated p53 protein levels following exercise and led to the hypothesis that p53 may be a stress-sensor in muscle that might engage repair programs in response to muscle stress and/or damage (12).

Although these studies succeeded in discovering novel gene expression in response to damaging resistance exercise, they each have limitations. First, several used animal models (2, 12), and there are likely species-specific differences in gene expression during recovery from muscle damage. Second, in the human studies (11, 30, 62), only time points within the first 24 h were assessed, and gene changes during the "second wave" of damage were not captured. In the present study, we used cDNA microarrays to screen for differentially expressed genes in human skeletal muscle during both early (3 h) and late (48 h) recovery from damaging exercise. Furthermore, given the large degree of intersubject response variability in exercise-induced gene expression (34, 35), we conducted our screens within each individual (i.e., repeated measures) at each time point to eliminate intersubject variance. We hypothesized that the aforementioned aspects of our study design would allow us to detect novel genes that responded to muscle damage, from which we could choose interesting candidate genes to confirm using real-time RT-PCR in a larger cohort of different subjects.

* These authors contributed equally to this work.

Address for reprint requests and other correspondence: M. A. Tarnopolsky, Dept. of Pediatrics, Rm. 4U4, McMaster Univ. Medical Center, 1200 Main St. W., Hamilton, Ontario, Canada, L8N 3Z5 (e-mail: tarnopol@mcmaster.ca).

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MATERIALS AND METHODS

Subjects. Fifteen healthy men (age: 23.4 ± 3.7 yr; height: 173 ± 10.7 cm; weight: 79.0 ± 4.6 kg; body mass index: 25.2 ± 2.9%) volunteered to participate in the study. We prescreened potential subjects to ensure that they had not regularly participated in resistance exercise within the preceding 6 mo and were healthy and fit to participate. Subjects were given an information sheet describing all of the testing procedures and they provided written consent to participate. The McMaster University Hamilton Health Sciences Human Research Ethics Board approved the project. The study conformed to the standards outlined in the *Declaration of Helsinki*.

Exercise protocol and tissue collection. We used an eccentric exercise protocol developed previously in our laboratory to induce muscle damage (3). Approximately 1 wk before the exercise protocol, subjects were given a familiarization session with the testing apparatus, a Biodex isokinetic dynamometer (System 3, Biodex Medical Systems, Ronkonkoma, NY). On the testing day, following a short warmup (10 min of light cycling), subjects were seated in the dynamometer, and their nondominant leg was strapped into a lever arm. We programmed the lever arm to extend the subject's leg to 150° of flexion (where 180° is full extension) at a moderate speed (30°/s), and then flex their leg to 90° of flexion at a fast speed (120°/s). Subjects did not have to contract during the extension phase. During the flexion phase, we instructed the subjects to attempt to extend their leg as much as possible (i.e., voluntary "maximal" contraction) against the descending lever arm throughout the entire range of motion. The entire test consisted of 30 sets of 10 repetitions, each set being separated by 1 min.

Before each tissue collection, subjects abstained from any form of physical exertion (within 72 h), avoided alcohol (within 48 h), ate their habitual diet (within 48 h), and abstained from caffeine (within 12 h). Each subject consumed a 355-kcal defined formula diet (67% carbohydrates, 18% protein, 15% fat; by mass) 2 h before each muscle biopsy and 2 h before the exercise bout; subjects did not eat anything else within 4 h of each biopsy. These nutritional controls ensured that the muscle damage would be the only variable that differentially affected mRNA expression from biopsy to biopsy (57). Muscle biopsies were taken from the vastus lateralis ~1–2 wk before (baseline), and 3 h and 48 h after exercise, in anatomically distinct sites of the same leg, as described (34). We chose these time points because they represent two distinct phases of recovery from muscle damage (15). Blood was drawn from the antecubital vein before, immediately, 3 h, and 48 h after exercise. Muscle and blood samples were processed and stored to be used for RNA extraction, enzyme analyses, and immunohistochemistry and histochemistry, as described previously (4, 34, 35, 41, 51, 52).

UV spectroscopy. We estimated sarcolemmal damage by measuring serum creatine kinase (CK) activity spectrophometrically using a commercially available kit (Procedure No. 47-UV, Sigma Diagnostics, St. Louis, MO). Triplicate estimates were used for all samples, and the intra-assay coefficient of variation was <7%.

Immunochemistry and histochemistry. We assessed ultrastructural damage histochemically by examining Z-band streaming (toludine blue stain), as described (51). Frozen 8-μm cross sections were processed for immunohistochemistry, as described (4), using the following primary antibodies (Dako Diagnostics, Glostrup, Denmark): mouse anti-human CD68 (M0814) for detection of macrophages, diluted 1:75; mouse anti-human myloperoxidase (MPO; M0748) for detection of neutrophils, diluted 1:300; mouse anti-human CD45RO (M0742) for detection of T-cells, diluted 1:200; and mouse anti-human CD20cy (M0755) for detection of B-cells, diluted 1:100. Stained samples were blindly examined and counted, as described previously (4).

Microarray and real-time RT-PCR analysis. Total RNA was extracted from muscle samples of four randomly chosen subjects, and amplified, labeled, and hybridized, as described (34, 35). Our cDNA microarrays were generated and processed, and the data were acquired, as described (35). Our arrays assessed 8,432 different genes that were randomly chosen from a human cDNA library, of which we were able to analyze ~7,000 after we removed PCR failures and those genes with nondetectable expression. Individual cDNA microarray comparisons were made for each subject (i.e., nonpooled) between each postexercise sample and the baseline sample. Unfortunately, one 48-h sample was lost during processing, and thus the 48-h data are from three subjects only. We used semiquantitative real-time RT-PCR from RNA extracted from the other 11 subjects to further analyze 19 gene changes from the microarray screen, using SYBR Green real-time chemistry and *β-2 microglobulin* as a housekeeping gene (34). We adhered to Minimum Information About Microarray Experiment criteria for all aspects of the study (6).

Statistical analysis. Given that we lost a sample during processing, we were unable to analyze our data using appropriate statistical analyses for microarray data, such as significance analysis of microarray (54). As such, given that we were most interested in further analyzing (using RT-PCR) genes that showed robust expression changes (which are most likely to be biologically relevant), we chose a conservative fold-change cutoff to define "differential gene expression" consisting of the following criteria: *1)* a mean fold change greater than 2; *2)* a minimum of three subjects having greater than twofold change. These data were then manually clustered into various categories, and a subset of 19 interesting candidate expression changes was further assessed using real-time RT-PCR. We analyzed real-time RT-PCR data using paired *t*-tests on the linear $2^{-\Delta CT}$ data set (33) and the muscle damage data using one-way repeated-measures ANOVAs, using computerized software (Statistica; Statsoft, Tulsa, OK).

RESULTS

Eccentric exercise induced muscle damage and inflammation in skeletal muscle. Eccentric exercise induced extensive Z-band streaming at 48 h (16-fold; $P < 0.001$; see Supplemental Fig. 1, *A–C* in the online version of this article), and serum CK activity was elevated at 3 h (2.2-fold; $P < 0.001$; see Supplemental Fig. 1*F*) and 48 h (3.9-fold; $P < 0.001$; Supplemental Fig. 1*F*). Hypercellularity was also observed (Supplemental Fig. 1, *D–E*), as neutrophils (14-fold; $P = 0.008$; Supplemental Fig. 1, *G–I*), and macrophages (4-fold; $P = 0.037$; Supplemental Fig. 1, *J–L*) invaded skeletal muscle 48 h postexercise, with no evidence of either T-cell or and B-cell infiltration. Together, these data confirm that our model was sufficiently stressful to induce muscle damage and inflammation in skeletal muscle.

DNA microarray screen identifies differential gene expression in skeletal muscle during recovery from damaging exercise. Our DNA microarray analyses detected that the mRNA expression of 113 genes was differentially increased 3 h posteccentric exercise, while that of 34 was decreased. At 48 h, the expression of 59 genes was differentially increased and that of 29 was decreased. We manually clustered many of these gene changes into "biological categories" that we felt were potentially relevant to recovery from and/or adaptation to damaging exercise and presented them in Table 1. The complete data set is freely available at GEO (accession no. GPL3457). Of note, a considerable number of these genes have been pulled out of similar screens in skeletal muscle after damaging exercise, including nuclear receptor 4A3 (*nor-1*), v-myc oncogene homologue (*c-myc*), cardiac ankryin repeat protein 1 (*CARP1*), cardiac/muscle LIM protein, heat shock protein 70 (*Hsp70*), *DnaJ*, *junD*, *IL-1 receptor*, *Nurr77*, and Nor1 (2, 11, 12, 62). Given that these expression changes have been observed by multiple groups, at different time points,

Table 1. *DNA microarray screen for differential gene expression during recovery from eccentric exercise*

Unigene Name	Unigene Cluster ID	3 h	48 h	Potential Relevent Function
Apoptosis				
BCL2 binding component 3	Hs.467020	5.2±5.8*	1.6±0.6†	proapoptotic protein
B-cell CLL/lymphoma 10	Hs.193516	5.0±3.1*	2.2±1.2†	antiapoptotic protein
Nuclear receptor 4A1	Hs.524430	2.4±0.3*	0.7±0.2†	may be proapoptotic
TNF receptor-associated factor 3	Hs.510528	0.5±0.1‡	0.5±0.2†	may be involved in NF-κB-mediated apoptosis
BH3 interacting domain death agonist	Hs.591054	0.1±0.1‡	n/a†	counters the protective effect of Bcl-2
Cholesterol and lipid homeostasis				
Caveolin 1, 22 kDa	Hs.74034	3.2±0.9*	1.5±0.5†	cholesterol transport across plasma membrane
SREBP 2	Hs.443258	3.2±0.4*	1.3±0.6†	transcription factor; controls cholesterol homeostasis
$PPAR_{\gamma}$	Hs.162646	2.4±0.2*	3.1±0.3*	transcription factor; fat/cholesterol metabolism
Stearoyl-CoA desaturase	Hs.558396	0.8±0.2†	4.8±3.4*	modifies lipids; lipogenesis
Low-density lipoprotein receptor	Hs.213289	2.3±1.4†	4.1±1.2*	transports LDL into cells
Insulin-induced gene 1	Hs.520819	1.6±0.1†	3.9±0.6*	negatively regulates SREBP
Acetyl-CoA acetyltransferase 2	Hs.571037	2.6±1.9†	3.5±0.8*	cholesterol absorption/synthesis
UDP-GCGT	Hs.304249	n/a†	3.3±0.7*	catalyzes synthesis of glycosylceramide
Growth				
Nuclear receptor 4A3 (nor-1)	Hs.279522	13±3.2*	0.6±0.7†	may activate cell proliferation
Tumor necrosis factor receptor 12A	Hs.355899	5.7±1.8*	n/a†	stimulates cell growth and angiogenesis
v-myc oncogene homolog (c-myc)	Hs.202453	5.7±3.1*	1.3±0.6†	immediate early gene; mediates growth
RhoGEF and pleckstrin domain 1	Hs.403917	5.4±3.0*	n/a†	may link the cytoskeleton to the cell membrane
MCIP 1	Hs.282326	3.8±1.5*	1.4±0.1†	inhibits calcineurin-mediated signaling
Protein kinase H11	Hs.400095	3.3±0.4*	2.4±1.3†	small HSP; induces cardiac hypertrophy
BAMBI	Hs.533336	3.1±1.6*	n/a†	interferes with TGFβ-mediated growth arrest
Retinoblastoma binding protein 6	Hs.188553	3.0±1.3*	n/a†	interacts with underphosphorylated pRB
Cbp/p300-interacting transactivator 2	Hs.82071	2.9±0.7*	2.0±0.4†	may induce cell proliferation; regulates HIF
PI_3kinase γ	Hs.32942	2.7±0.2*	n/a†	mediates growth signaling in muscle
AT rich interactive domain 5B	Hs.535297	2.6±0.5*	1.8±0.9†	DNA-binding protein
Cyclin-dependent kinase inhibitor 3	Hs.84113	2.1±0.4*	2.0±1.1†	inhibits cdk2 and prevents G1 to S transition
Activin A receptor, type IIA	Hs.470174	2.5±0.7*	n/a†	dimeric growth and differentiation factor
Collagen, type VI, alpha 2	Hs.420269	2.5±0.7*	1.9±0.6†	involved in collagen fibrinogenesis
Zinc finger and BTB domain (32)	Hs.99430	16±6.5*	11±7.4*	interacts with GATA-2; satellite cell activation?
Cardiac ankyrin repeat protein 1	Hs.448589	9.7±3.5*	14±5.0*	titin filament-based protein; mechanical signaling
Keratin 5	Hs.433845	5.2±2.9*	8.1±3.5*	interacts with muscle specific intermediate filaments
Nuclear factor I/C	Hs.170131	3.1±1.2*	3.4±2.8*	activates transcription and replication
Polymerase ε	Hs.524871	3.1±1.3*	2.7±1.3*	DNA replication and repair
GATA binding protein 5	Hs.352250	2.6±0.6*	2.3±0.3*	may be involved in cell differentiation
Dystrophia myotonica-protein kinase	Hs.631596	1.4±0.3†	4.4±1.7*	mutations cause myotonic dystrophy; myogenesis?
Nitric oxide synthase 3	Hs.438823	2.1±1.2†	4.0±2.6*	produces nitric oxide; angiogenesis?
Cardiac/muscle LIM protein	Hs.83577	1.8±0.3†	3.6±2.2*	mechanical sensor; stabalizes structural proteins
Son of sevenless homolog 1	Hs.278733	n/a†	2.9±1.0*	activates RAS; may regulates cell growth?
Glutamyl-prolyl-tRNA synthetase	Hs.497788	1.1±0.1†	2.6±0.2*	participates in translation; tRNA synthetase
LSM3 homolog	Hs.111632	n/a†	2.6±0.4*	involved in premRNA splicing
Capping protein, muscle Z-line, α1	Hs.514934	n/a†	2.4±0.2*	interacts with α-actinin; promotes sarcomerogenesis
Chromatin assembly factor 1B	Hs.75238	0.4±0.3†	0.3±0.1‡	chromatin assembly in DNA replication and repair
Microtubule associated protein like 1	Hs.12451	0.8±0.3†	0.3±0.1‡	may modify microtubules
ADAM metallopeptidase domain 19	Hs.483944	0.8±0.4†	0.2±0.1‡	proteolytic processing; involved in synaptogenesis
Microfibrillar associated protein 5	Hs.512842	1.1±0.4†	0.2±0.1‡	component of the elastin-associated microfibrils
Coagulation factor II receptor	Hs.482562	n/a†	0.2±0.1‡	role in platelet activation and vascular development
Transmembrane 4 superfamily (7)	Hs.437594	0.5±0.1‡	0.4±0.1‡	may have role in cell development, activation, growth
Metabolism				
Mitochondrial ribosomal protein L32	Hs.50252	3.3±1.0*	1.3±0.5†	involved in mitochondrial protein synthesis
Pyruvate dehydrogenase kinase, 4	Hs.8364	2.7±0.6*	n/a†	inhibits pyruvate dehydrogenase complex
Mitochondrial ribosomal protein L2	Hs.55041	6.8±3.5*	5.0±7.0*	mitochondrial translation
Muscle glycogen phosphorylase	Hs.154084	1.2±0.7	0.3±0.1‡	key enzyme in glycogen breakdown
Pyruvate dehydrogenase kinase, 1	Hs.470633	n/a	0.2±0.1‡	inhibits the pyruvate dehydrogenase complex
Pyruvate dehydrogenase kinase, 2	Hs.256667	0.8±0.2	0.2±0.1‡	inhibits the pyruvate dehydrogenase complex
Thyroid hormone receptor, alpha	Hs.724	0.2±0.1	0.4±0.2‡	nuclear hormone receptor for triiodothyronine
Proteolysis				
Ubiquitin specific protease 2	Hs.524085	7.4±1.9*	0.9±0.4†	E4 ubiquitin recycling enzyme; involved in UPS
Cullin 1	Hs.146806	4.4±0.4*	1.6±0.1†	E3 ubiquitin ligase; involved in UPS
Ubiquitin-activating enzyme E1-like	Hs.16695	1.9±0.6†	2.3±0.1*	UPS-mediated proteolysis
Proteasome 26S subunit 3	Hs.12970	0.4±0.1‡	2.0±0.5†	regulatory subunit of the 26 proteasome
Ubiquitin specific protease 1	Hs.35086	0.3±0.1‡	n/a†	protein degradation
E2 ubiquitin conjugating enzyme	Hs.529420	1.7±0.2†	0.3±0.1‡	ctalyzes ubiquitin attachment to proteins
Stress management				
Heat shock 70 kDa protein 1-like	Hs.558337	10±5.9*	1.3±0.4†	manages heat and protein stress
DnaJ homolog A1	Hs.445203	5.3±1.6*	1.4±0.4†	manages heat and protein stress
jun D proto-oncogene	Hs.2780	3.8±1.9*	1.1±0.1†	immediate early gene; mediates stress response
Interleukin 1 receptor, type I	Hs.557403	2.6±1.3*	2.4±2.1†	participates in the inflammatory response

Continued

Table 1.—*Continued*

Unigene Name	Unigene Cluster ID	3 h	48 h	Potential Relevent Function
Tyrosyl-DNA phosphodiesterase 1	Hs.209945	12±3.2*	6.7±8.2*	repairs DNA damage, including oxidant-induced
DnaJ homolog B2	Hs.77768	7.4±0.8*	4.5±0.7*	manages heat and protein stress
Heat shock transcription factor 4	Hs.512156	6.0±3.9*	4.0±3.1*	activates heat-shock genes?
v-abl homolog 1	Hs.431048	3.3±4.4*	32±15*	immediate early gene
Proenkephalin	Hs.339831	3.1±0.8*	3.4±0.9*	manages pain perception
DnaJ homolog, C1	Hs.499000	1.8±0.5†	3.0±1.0*	manages heat and protein stress
GADD-inducible γ	Hs.9701	0.8±0.1†	0.4±0.1‡	mediates stress response to DNA damage
Metallothionein 1F	Hs.513626	0.4±0.3†	0.3±0.1†	protection against oxidant and metal stress
Metal transcription factor 1	Hs.591505	n/a†	0.2±0.2†	transcriptional activator of metallothioneins
Transcription				
Splicing factor 1	Hs.502829	8.2±2.1*	n/a†	spliceosome assembly
RNA polymerase III subunit RPC8	Hs.643610	2.6±0.4*	4.3±1.5*	transcribes DNA
Kruppel-like factor 2	Hs.107740	0.4±0.1‡	1.2±0.4†	activates transcription
Transport				
Bicaudal D homolog 1	Hs.505202	3.7±2.5*	n/a†	involved in Golgi-ER transport
NMDA receptor	Hs.558334	20±11*	7.9±8.9*	glutamate-gated ion channel w/t high Ca^{2+} permeability
WNK lysine deficient protein kinase 1	Hs.356604	1.0±0.3†	5.9±1.5*	involved in sodium and chloride ion transport
Potassium channel, V1	Hs.13285	1.8±0.3†	4.1±1.3*	potassium transport across membranes
Solute carrier 6, member 8	Hs.540696	n/a†	3.2±0.8*	creatine transport across membranes
Glycoprotein M6A	Hs.75819	n/a†	2.9±0.1*	growth factor-gated Ca^{2+} channel
Vacuolar protein sorting 41	Hs.592184	1.3±0.4†	2.8±0.3*	required for vacuolar assembly and traffic
Voltage-gated calcium channel, β1	Hs.635	1.3±0.3†	2.6±0.7*	calcium transport across membranes
Cholinergic receptor, nicotinic, α3	Hs.89605	0.8±0.5†	2.6±0.1*	acetylcholine signaling
Miscellaneous				
MAP kinase kinase kinase 5	Hs.186486	n/a†	2.8±0.2*	phosphorylates and activates MAP kinase kinases
MAP kinase kinase 1	Hs.145442	1.3±0.1†	2.6±0.6*	activates ERK1 and ERK2 MAP kinases
Gua-nucleotide exchange factor 7	Hs.508738	n/a†	2.3±0.3*	guanine nucleotide exchange factor

*, differential increase; †, no change; ‡, differential decrease. For the "growth" category, we included all genes that might be related to muscle growth or remodeling, including those that encode for proteins involved in muscle structure, protein synthesis, cell cycle regulation, satellite cell activation, and ECM and vascular growth or remodeling. BCL2, B-cell lymphoma protein 2; CLL, chronic lymphocytic leukemia; BH3, BCL-2 homology 3; SREBP2, sterol response element binding protein 2; PPARγ, peroxisome proliferator-activated receptor γ; CoA, coenzyme A; UDP-GCGT, UDP-glucose ceramide glucosil transferase; RhoGEF, Rho-specific guanine nucleotide exchange factor; MCIP 1, myocyte-enriched calcineurin interacting protein 1; BAMBI, BMP and activin membrane-bound inhibitor; BTB, bric-a-brac, tramtrack, broad; LIM, Lin-11, Is-1, Mec-3; LSM3, Like SM3; ADAM, a disintegrin and a metalloproteinase; LDL, low-density lipoprotein; HSP, heat shock protein; TGF, transforming growth factor; UPS, ubiquitin proteasome system; ER, endoplasmic reticulum; MAP, mitogen-activated protein; UDP, uridine diphosphate; BMP, bone morphogenic protein. Note: Not all differentially expressed genes are shown. For a complete data set, visit www.ncbi.nlm.nih.gov/geo/, accession GPL3457.

from several muscles, following various exercise protocols, and in multiple species strongly suggests that they are biologically relevant gene changes, likely involved in recovery from and/or adaptation to damaging exercise. Furthermore, the considerable overlap between our data set and those from previously published screens speaks strongly toward the validity of our screen. This being said, given that our small sample size precluded us from running appropriate, microarray-specific statistics on the data, we decided to use the microarray data entirely as an initial screen for interesting and robust gene changes, from which we chose 19 to further analyze using real-time RT-PCR.

Table 2. *Primer pairs and amplicon size for real-time RT-PCR analyses*

Gene Name	Left Primer	Right Primer	Amplicon (nt)
Acetyl-CoA acetyltransferase 2	aagccatgtccaagctaag	tgtaagcccacgtttatca	122
β2-Microglobulin	atgagtatgcctgccgtgtga	ggcatcttcaacctccatg	101
Capping protein, muscle Z-line, α1	ccaacggcttctgtactgttt	gctgtaggtggtgtgatggt	148
Cardiac ankyrin repeat protein 1	gcggatctcaacatcaagaa	tgtcgtttgcctcagaatgt	146
Caveolin 1, 22 kDa	cagtgcatcagccgtgtcta	tcccaacagcttcaaagagtg	70
DnaJ homolog B2	ggagccaggttgcatttatt	ccaagtcctccttccttgac	122
MCIP 1	caaccgtgcaacgtgtaact	ccgatcccaaacacctaaac	147
Protein kinase H11	agaaggtggcattgtttcta	catctcaggtacaggtgact	206
Interleukin 1 receptor, type I	ttatgcctcatgctgactt	cttcccaagccctctactct	145
Insulin induced gene 1	taaccacgccagtgctaaat	ccacttctggaacgatcaaa	108
jun D proto-oncogene	cgcctggaagagaaagtga	ttgacgtggctgaggactt	116
Low density lipoprotein receptor	tgcctcatccaccaatctcta	aaacccacctcgaaagacact	149
Pyruvate dehydrogenase kinase, 4	caaggatgctctgtgatcagtattattt	tgtgaattggttggtctggaaa	93
PPARγ	agaagctgttggcggagat	cagcgggaaggactttatgta	113
Stearoyl-CoA desaturase	aagaagcaagggcaagaac	tcagcagggtttgtggtatc	121
SREBP 1c	cactgaggcaaagctgaataaa	tgttgcagaaagcgaatgtagt	69
SREBP 2	ctctgaccagcacccacact	cacaccatttaccagccataag	118
c-myc	ccagaggaggaacgagctaa	ttggacggacaggatgtatg	130

Real-time RT-PCR analyses reveal novel differential gene expression in skeletal muscle during recovery from damaging exercise. An unexpected finding from the DNA microarray screen was that eccentric exercise induced the expression of a number of genes involved in cholesterol and lipid homeostasis (Table 2). As such, we analyzed several of these gene changes using real-time RT-PCR (Fig. 1) and found a rapid (3 h) and significant increase in the expression of *SREBP-2* (1.4-fold, $P = 0.023$), but not *SREBP-1c* (1.2-fold, $P = 0.28$), as well as delayed (48 h) and significant increases in several SREBP-2 gene targets, such as *ACAT-2* (2.2-fold, $P = 0.018$), *insig-1* (1.4-fold, $P = 0.003$), and *SCD* (2.5 fold, nonsignificant trend, $P = 0.10$) (Fig. 1) (36). SREBP-2 is a transcription factor that regulates cholesterol homeostasis and lipogenesis (7). ACAT-2 and SCD participate in cholesterol synthesis/transport and phospholipid synthesis, respectively, while *insig-1* is the major negative regulator of SREBP-2 (9, 50, 56, 59). The expression of each of these downstream targets was elevated to a relatively similar degree (1.4–2.-fold), suggesting coordinate induction. Additionally, the expression of both the interleukin-1 (*IL-1*) receptor (3.2-fold at 3 h, $P = 0.001$) and *PPARγ* (1.6- fold at 48h, $P = 0.03$, data not shown), known regulators of SREBP-2 (19, 24, 28), was increased after exercise (Fig. 1). Collectively, these expression changes suggest a transcriptional response centered on SREBP-2 that would lead to cholesterol synthesis and phospholipid modification. It should be noted that we were unable to confirm gene changes observed on the microarray screen for both *caveolin 1* and the *LDL receptor* (data not shown).

As predicted, the DNA microarray screen picked up a large number of differentially expressed genes involved in protein synthesis pathways, satellite/stem cell activation, cell cycle regulation, and stress management, and those encoding structural proteins (Table 1). As mentioned, a considerable number of these changes have been previously reported by others, and for technical reasons (initial validation of the array data), we chose several of these to validate using real-time RT-PCR (Fig. 2). As expected, eccentric exercise induced the expression of *CARP1* (12-fold at 3 h, $P = 0.013$; 13-fold at 48 h, $P = 0.022$), *DNAJB2* (1.7-fold at 3 h, $P = 0.038$; 1.7-fold at 48 h, $P = 0.031$), *JunD* (3.8-fold at 3 h, $P = 0.008$), *c-myc* (10.6-fold at 3 h, $P = 0.006$), and *PDK4* (6.4-fold at 3 h, $P = 0.025$). More importantly, however, a number of the observed gene changes have not previously been reported, and we chose to

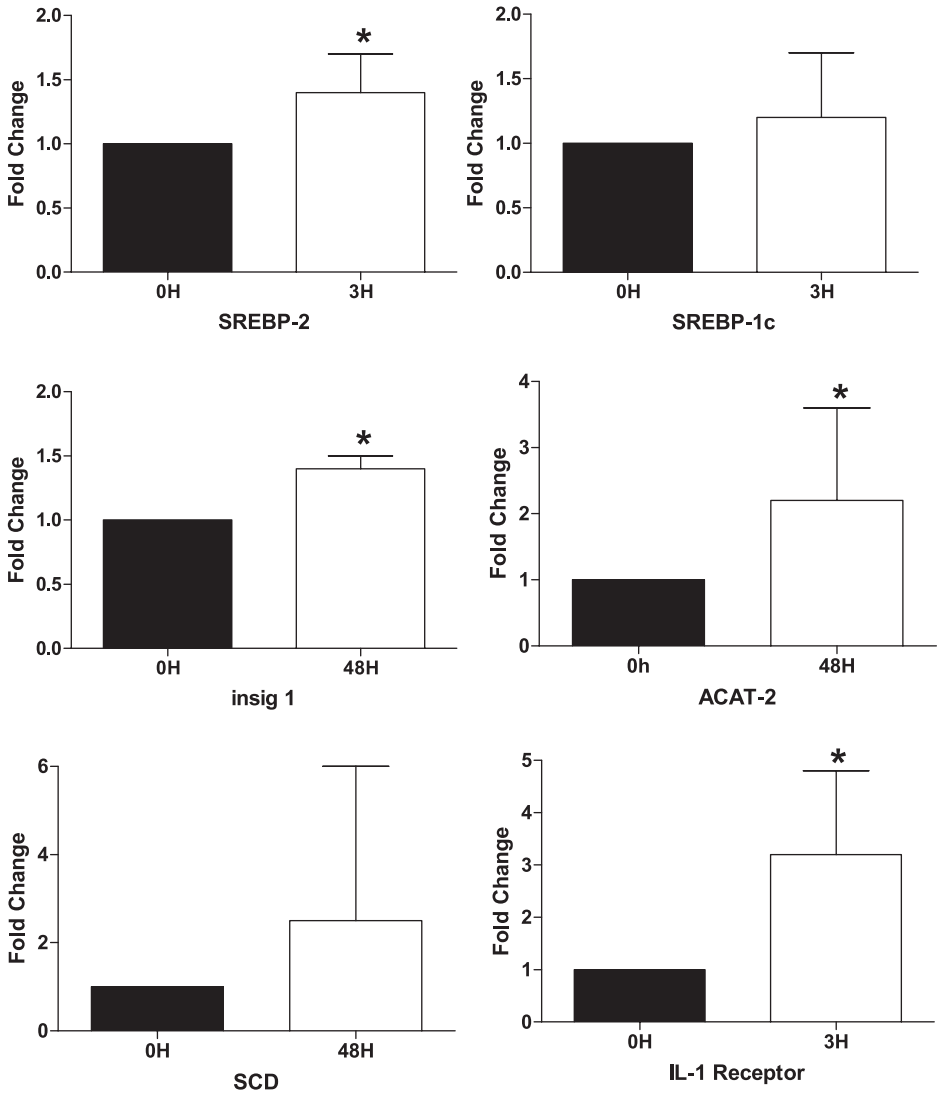

Fig. 1. Eccentric exercise induces mRNA expression of genes involved in lipid and cholesterol homeostasis. Graphic representation of mRNA expression data for the indicated genes at either 3 h or 48 h after eccentric exercise, as assessed using real-time RT-PCR. *Statistically significant changes ($P < 0.05$).

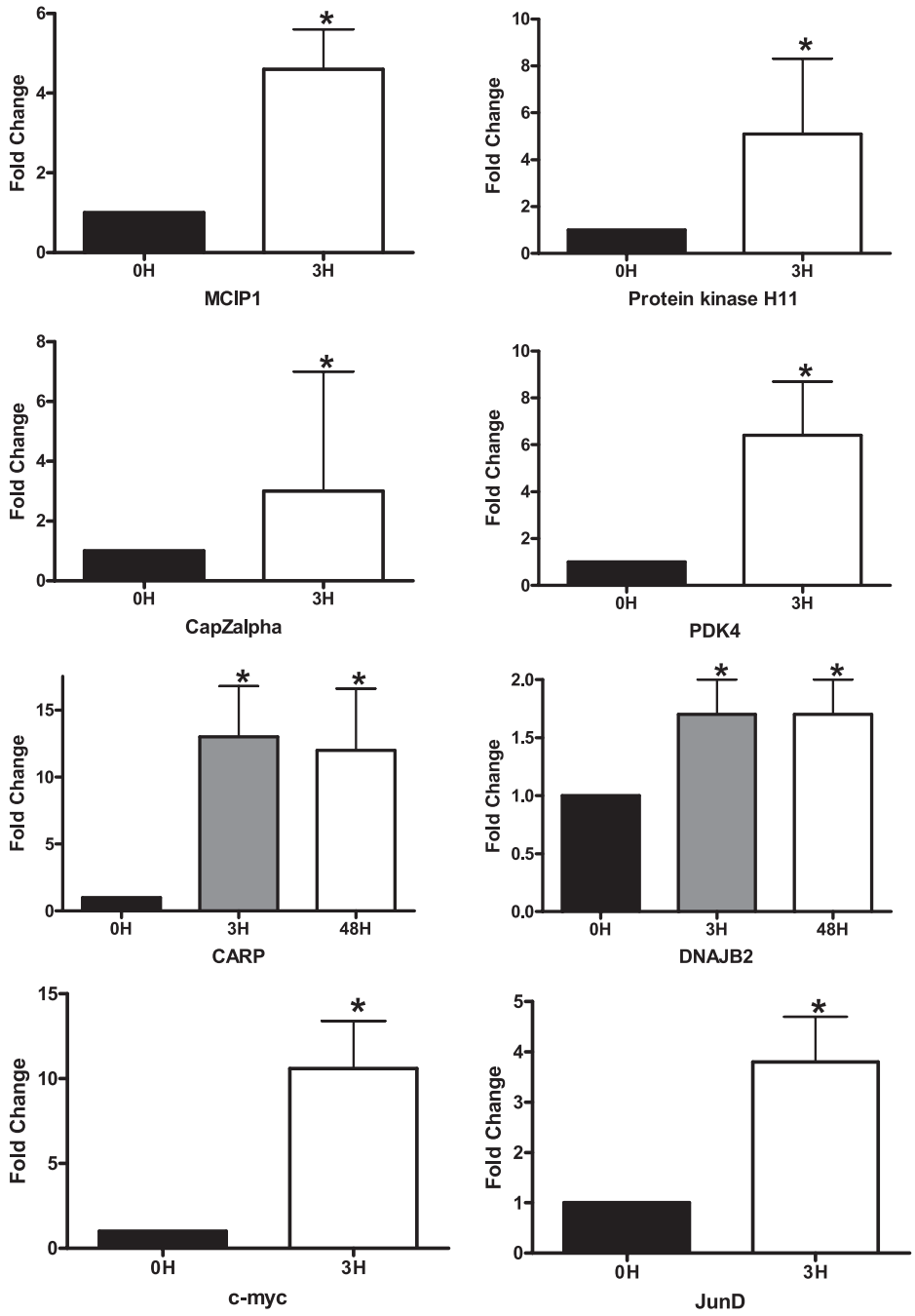

Fig. 2. Eccentric exercise induces mRNA expression of genes involved in stress management and growth. Graphic representation of mRNA expression data for the indicated genes at either 3 h or 48 h after eccentric exercise, as assessed using real-time RT-PCR. *Statistically significant changes ($P < 0.05$).

further analyze several that, based on what is known of their biology in the literature, we thought might be particularly relevant to recovery from and/or adaptation to skeletal muscle damage. These analyses demonstrated that eccentric exercise led to elevated expression levels of *protein kinase H11* (5.1-fold at 3 h, $P = 0.002$), a small heat shock protein involved in cardiac hypertrophy (14), *MCIP1* (4.6-fold at 3 h, $P = 0.009$), which inhibits calcineurin signaling and may be involved in fiber-type determination (45), and CapZα (3.0-fold at 48 h, $P = 0.001$), which interacts with α-actinin and is involved in sarcomerogenesis (40). Taken together, these expression changes describe a program of muscle remodeling that is rapidly induced and persists throughout the 48-h time course. Here, we have identified three novel expression changes that may be important in this process.

DISCUSSION

Herein, we have presented real-time RT-PCR data for differently expressed genes in human skeletal muscle during recovery from acute muscle damage. The genes analyzed were selected from a preliminary DNA microarray screen conducted on a subset of the participants in the study and were chosen either as technical controls for the exercise and microarray protocols (i.e., genes that had previously been shown to respond to eccentric exercise and were detected by our microar-

ray analyses), or more importantly, because to our knowledge, there are no previous reports of their involvement during recovery from eccentric exercise, and their known biological function suggests that they may play a role in recovery from and/or adaptation to damaging exercise (Fig. 3).

The two most striking observations were *1*) the novel set of genes were involved in cholesterol and lipid homeostasis and *2*) the novel set of genes were involved in stress management and growth, which were induced in response to eccentric exercise. First and foremost, damaging exercise induced a rapid (3 h) increase in *SREBP-2* expression, followed by a delayed increase in three known SREBP-2 downstream gene targets, *ACAT-1*, *insig-1*, and *SCD*. SREBP-2 is a member of the SREBP family of basic helix-loop-helix transcription factor that normally resides as an inactive precursor protein embedded in the endoplasmic reticulum membrane (7). Upon appropriate stimulation, SREBP-2 undergoes posttranslational cleavage and activation, migrates to the nucleus, and stimulates the transcription of numerous gene targets involved primarily in cholesterol and lipid homeostasis. SREBP-2 also transcriptionally activates itself, providing a strong feed-forward mechanism for amplifying SREBP-2-mediated responses. Given that *SREBP-2* expression was elevated early (3 h) and the expression of its downstream targets was elevated late (48 h), suggests that a linear SREBP-2-mediated transcriptional program was engaged in skeletal muscle in response to eccentric exercise. It is noteworthy that the expression of the *IL-1 receptor*, a known regulator of SREBP-2, was also elevated at the early time point.

What might the biological role of an SREBP-2 transcriptional response in skeletal muscle after damaging exercise be? Although we can only speculate, the results of several recent studies lead us to believe that SREBP-2 activation may induce a membrane biosynthetic program in response to muscle damage. Demoulin et al. (16) analyzed global mRNA expression after stimulating fibroblast growth and observed an SREBP-1/2 transcriptional response containing many overlapping genes to those found in our study. Importantly, these authors demonstrated that SREBP-1/2 activation induced de novo membrane biosynthesis to accommodate the growing fibroblasts (16). Furthermore, SREBP-1/2 activation was regulated by the PI_3 kinase-Akt signaling axis, which is an integral signaling pathway for skeletal muscle growth (25). In direct support of this, genetic Akt activation in human retinoic pigment epithelial cells was shown to induce SREBP-1/2 protein accumulation, as well as key enzymes involved in cholesterol and fat biosynthesis and de novo membrane biosynthesis (44). Finally, Castoreno and colleagues (10) studied cell cycle-independent de novo membrane biosynthesis and demonstrated that it too is regulated by an SREBP-1/2-mediated transcriptional program. During phagocytosis in human embryonic kidney 293 cells, bilayer synthesis was accompanied by SREBP-1/2 activation and transcriptional upregulation of numerous SREBP-regulated lipogenic proteins. Importantly, SREBP inhibition by insig-1 abolished phagocytosis-induced transcription and membrane biogenesis. Taken together, these recent studies demonstrate that SREBP family transcription factors are central regulators of membrane biosynthesis in mammalian cells. Given that eccentric exercise induces membrane damage and is a potent stimulus for muscle cell growth, we speculate that SREBP-2 activation may engage a transcriptional program for de novo membrane biosynthesis in skeletal muscle.

Eccentric exercise also affected a number of genes encoding structural and regulatory proteins that are likely involved in managing stress within the muscle, strengthening the muscle architecture or inducing muscle growth. Within this cluster of genes, we found three to be particularly interesting, as *1*) they are highly expressed in skeletal muscle; *2*) their known function suggests that they may be important mediators of muscle integrity, stress management, and/or growth; and *3*) they have not been previously examined in skeletal muscle following a damaging bout of exercise.

Protein kinase H11 is a member of the small heat shock protein family and is highly expressed in cardiac and skeletal

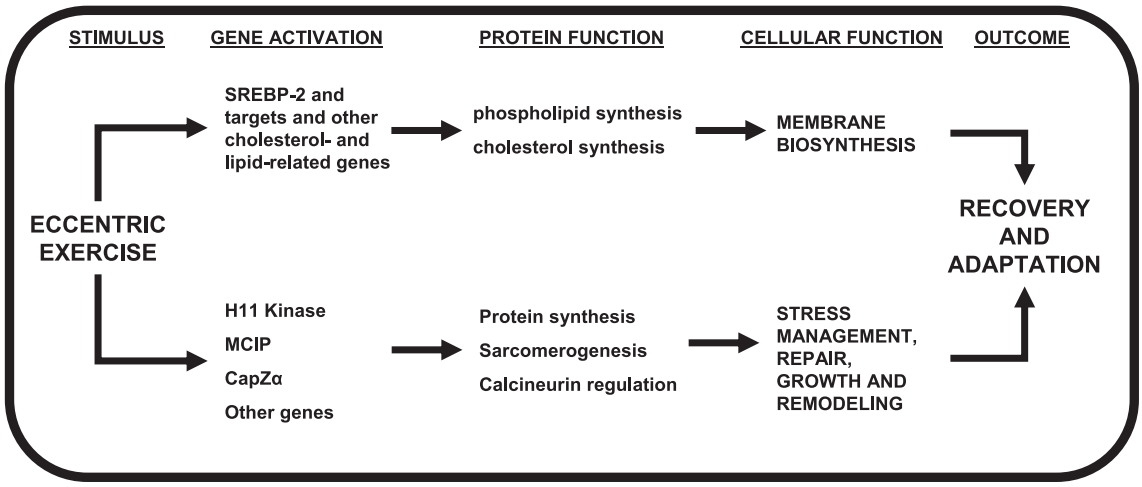

Fig. 3. Schematic representation of the potential biological significance of the gene expression changes observed after eccentric exercise. *Upper arm*: eccentric exercise activated sterol response element binding protein 2 (SREBP-2) and numerous SREBP-2 gene targets involved in cholesterol and lipid regulation. Collectively, these expression changes suggest a transcriptional program geared toward increasing cholesterol and lipid synthesis and regulation in skeletal muscle. This program may represent a stimulus for de novo membrane biosynthesis in skeletal muscle, which may be involved in recovery from membrane damage or adaptation to growth signals. *Lower arm*: eccentric exercise activated a large number of genes involved in protein content regulation, sarcomere synthesis, and stress regulation. These expression changes suggest a transcriptional stimulus for muscle cell stress management and repair, as well as growth and remodeling. Collectively, these programs may be involved in recovery from and adaptation to the eccentric exercise bout.

muscle (27). Recently, mutant H11 was shown to be responsible for distal motor neuropathy (26). H11 has strong chaperone activity, physically interacts with other important heat shock proteins in muscle (e.g., HSP27), and protects cells from thermal and oxidative stress (14, 38, 53). Accordingly, elevated *H11* expression posteccentric exercise may have been in response to thermal, protein, or oxidative stress. H11 has also been shown to mediate cardiac hypertrophy in vitro and in vivo. Depre et al. (17) demonstrated increased *H11* mRNA expression and protein content in multiple models of cardiac hypertrophy. Furthermore, forced H11 expression in cultured cardiac myocytes resulted in elevated protein/DNA ratio, and hearts from transgenic H11-overexpressing mice were heavier and had increased myocyte cross-sectional area. Importantly, both Akt and $p70^{S6}$ kinase were activated in transgenic hearts in an H11 dose-dependent manner. Given that these two kinases are activated in skeletal muscle during hypertrophy (1, 5) and their activation strongly induces skeletal muscle protein synthesis (25), H11 kinase may represent a novel mediator of hypertrophy in skeletal muscle.

MCIP1 is a member of the MCIP family of calcineurin inhibitors (45) and possibly a marker of calcineurin activation (58). MCIP1 is activated by oxidative and calcium stress (31) and is able to provide short-term protection from these stressors (21). These effects may be mediated by MCIP1-induced activation of superoxide dismutase 1 and repression of calcineurin, respectively (20). Given that oxidative stress and intracellular calcium dysregulation are known sequelea of eccentric exercise, it is possible that *MCIP1* is a stress-responsive gene. Alternatively, MCIP1 may regulate intracellular calcineurin signaling in muscle (47). MCIP1 is transcriptionally activated by calcineurin-mediated NF-AT signaling (58) and is a strong allosteric inhibitor of calcineurin (45). As such, MCIP1 is thought to participate in a regulated negative feedback loop that controls calcineurin-mediated signal transduction (47). This pathway has been shown to be physiologically important in the heart, in which MCIP1 regulates cardiac hypertrophy mediated by calcineurin signaling (46, 55). The physiological role of calcineurin in skeletal muscle is controversial. Although originally thought to mediate hypertrophy (37), it now appears that calcineurin's role may be to regulate muscle fiber type (42), as it has been shown to activate slow-fiber gene expression and mediate fast-to-slow fiber-type conversion (13, 39). Thus, although MCIP1 may be involved in regulating the hypertrophy response after eccentric exercise, it is more likely involved in preventing a fast-to-slow fiber-type conversion.

*CapZα*1 is an alpha-actin capping protein in striated muscle (32). This protein regulates thin-filament dynamics in the sarcomere and anchors the thin filament to the Z-disc by directly interacting with α-actinin (40). CapZ is thought to be particularly important during sarcomerogenesis, in which it plays a crucial role in aligning the thin filament with the Z-disc (40, 48, 49). Recently, sarcomerogenesis was eloquently demonstrated after a bout of eccentric exercise in humans (60, 61), although the molecular details were not definitely elucidated. Given that eccentric exercise induced a delayed increase in *CapZα1* mRNA expression at the same time that extensive sarcomere disruption was observed, this protein may be involved in the repair and/or assembly of sarcomeres posteccentric exercise.

Although not confirmed by RT-PCR, there were a number of differentially expressed genes whose proteins are involved in various aspects of proteolysis (Table 1). As protein degradation is likely an important aspect of damage resolution prior to remodeling and adaptation, we expected to see gene changes in this area. Somewhat unexpectedly, many of the genes were upregulated, while many others were downregulated, highlighting the complexity in the regulation of proteolysis following damaging exercise. Future experiments are clearly warranted to evaluate the role of these proteins in adaptation to and/or recovery from muscle damage.

Technical considerations. Unless using an in situ technique, muscle biopsy studies are limited in that it is not possible to determine whether an observed change occurred in myofibers or other cell types within muscle (e.g., endothelial cells, fibroblasts, and resident macrophages). In our opinion, given that *1*) muscle cells/nuclei are the major mRNA contributor to a muscle biopsy sample and *2*) using immunohistochemistry, we did not observe an influx of inflammatory cells until 48 h, most of our changes likely occurred in myofibers. However, we cannot rule out the possibility that some of these gene changes occurred in nonmyogenic cells. Regardless, these changes occurred in skeletal muscle tissue in response to muscle damage and likely represent important transcriptional events for recovery from and adaptation to muscle damage. Future research using conventional molecular biology techniques will hone in on the cell type responsible for these gene changes.

A second limitation in this study is the small sample size used for the microarray analyses, which likely prevented us from detecting many gene changes that occurred. Clearly, future studies are warranted using this technology in a larger cohort of subjects.

Perspectives and Significance

The major finding in this study is that eccentric exercise induced an SREBP-2-mediated transcriptional response that we speculate are involved in *de novo* membrane biosynthesis in skeletal muscle. This response may have been activated by sarcolemmal damage induced by eccentric exercise, as the SREBP pathway has previously been shown to act as a "membrane sensor" (18). Alternatively, it may have been activated by the growth stimulus of eccentric exercise (16), as the sarcolemma must expand to accommodate a growing muscle fiber. Skeletal muscle remodeling after a damaging and/or hypertrophic stimulus involves all major components within the muscle, and this study lays the groundwork for future research examining sarcolemmal remodeling in response to such stimuli. Elucidating the signaling pathways that link muscle damage and/or the stimulus for muscle growth to sarcolemmal remodeling will be particularly important, especially if these turn out to be shared pathways between multiple repair and/or growth responses. Finally, given that sarcolemmal damage is pervasive in many types of muscle diseases, in particular, the dystrophinopathies, understanding the molecular regulation of *de novo* membrane biosynthesis in response to muscle damage may have useful clinical implications.

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