Differential genomic responses in old vs. young humans despite similar levels of modest muscle damage after resistance loading

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Postnatal skeletal muscle repair and regeneration following acute injury (e.g., high-intensity exercise bout, trauma, surgery, drug-induced necrosis) involves a complex array of coordinated activities that is incompletely understood. On the other hand, it is widely accepted that muscle progenitor cell [satellite cell (SC)] recruitment is a key component of the process, indicating that at least some aspects of developmental (secondary) myogenesis are recapitulated during postnatal muscle regeneration (35, 54). The nominally quiescent SCs reside just outside of the myofiber sarcolemma beneath the basal lamina and are thus architecturally positioned to respond rapidly to cues from the circulation, the extracellular matrix, and secreted factors exiting nearby myofibers (28). The appropriate regeneration response involves SC proliferation; progression along a myogenic lineage to differentiated, fusion-competent myoblasts; and fusion to injured, repairing myofibers as nuclear donors or, with overt damage (e.g., trauma, surgery), myoblasts fuse with one another to form multinucleated myotubes that then develop into replacement myofibers. These SC functions and their molecular regulation are described in a number of excellent reviews (10, 35, 51, 54). Although the precise mechanisms are not fully understood, SC function is clearly impaired with advancing age as shown in both human (8) and rat (4) primary SCs. Furthermore, in vivo aging rodent studies have demonstrated that SC recruitment is impaired during atrophy countermeasures (23) and SC-mediated regeneration following injury is less effective (13, 14), which is at least partially caused by the aging SC niche (14). Concurrent to the initiation of regenerative mechanisms after skeletal muscle injury is an induction of inflammatory and stress responses (19, 27, 55) including an infiltration of macrophages (2, 12). These responses may “jump-start” regeneration; however, prolonged or hyperactivated proinflammatory cytokine signaling impedes the process (42). Furthermore, inhibiting the conversion of invading macrophages to the anti-inflammatory M2 phenotype impairs muscle regeneration (52). A better understanding of the principal mechanisms driving skeletal muscle regeneration is essential to fully appreciate key alterations in the aging muscle that cause disruption.

Microarray analysis allows for comprehensive and simultaneous assessment of all skeletal muscle transcripts and provides a unique opportunity to identify age-dependent changes in the skeletal muscle transcript profile after a stressor or injury stimulus [e.g., unaccustomed resistance loading (RL)] that might have otherwise been unidentified. While a few transcript profiling studies of resistance exercise-mediated changes have been published (31, 38, 41, 50, 60), none has tested differential age responses to contraction-mediated damage across the entire

While skeletal muscle is recognized as an exceptionally plastic tissue, aging skeletal muscle demonstrates an impaired capacity for regeneration when exposed to the same injury stimulus as young muscle, as shown definitively in rodent models (13, 14, 39). This creates a concern for older adults as they transition toward frailty and/or attempt to recover from surgery, because sarcopenia (i.e., age-related skeletal muscle atrophy) is exacerbated in these patients and they are unable to restore affected muscle mass to presurgery levels despite intensive physical intervention (48).
transcriptome, because these studies were conducted in younger men only (38, 60), were long-term training studies (41, 50), or did not assess changes in the whole transcriptome (31).

The purpose of this study was therefore to determine, by genomewide microarray analysis including functional gene networks and pathways analysis, whether young and old adults respond differentially to a standardized bout of unaccustomed RL, sufficient to induce moderate muscle damage. Our overarching hypothesis was that any age differences in the transcriptome-level response may reveal key factors responsible for the muscle regeneration impairment among the old.

**METHODS**

Forty-six young and older men and women were studied (subject characteristics in Table 1). Health history and physical activity questionnaires were completed by all participants during screening. Participants in the older group also passed a comprehensive physical exam and a diagnostic, graded exercise stress test with 12-lead ECG. Subjects were excluded for any musculoskeletal or other disorders that might have affected their ability to complete the RL bout and testing for the study; obesity (body mass index (BMI) > 30.0 kg/m²); knee extensor resistance training within the past 5 yr; and treatment with exogenous testosterone or other pharmacological interventions known to influence muscle mass or muscle recovery. The study was approved by the Institutional Review Boards of both the University of Alabama at Birmingham (UAB) and the Birmingham Department of Veterans Affairs (VA) Medical Center. All subjects provided written informed consent before participation.

**Unaccustomed resistance loading bout.** Mechanical overload of the knee extensor muscle group was accomplished by a standardized RL bout designed specifically to induce moderate myofiber wounding, because subjects were untrained and unaccustomed to the RL. The RL bout consisted of 9 sets of ~10 repetitions of bilateral, concentric- eccentric knee extension contractions against a constant external load on a conventional weight-stack knee extension machine. Subjects were instructed and encouraged throughout to perform the concentric phase of each repetition as rapidly as possible, followed by their best attempt to control the eccentric lowering phase. The external resistance set for each subject was equivalent to ~41,000 gene transcripts and the standard single-color array protocol at Agilent’s Center for Excellence and Cogenics. For standardization, pre- and post-RL samples within each subject were analyzed in the same batch and each batch included samples from both young and old subjects.

**Immunoblotting.** Standard immunoblotting was performed with established methods in our laboratory (3, 33) on samples from 32 subjects (16 young, 16 old); groups were balanced by sex (8 men, 8 women per age group). Muscle protein lysate was extracted from frozen muscle samples (average 30–35 mg) as detailed previously (3, 32, 33). Protein concentrations were determined by the bichinchoninic acid (BCA) technique with bovine serum albumin as a standard. Samples were run on 4–12% Bis-Tris (Invitrogen) SDS-PAGE gel matrices with 30 μg of total protein loaded into each well, which was determined as ideal by preliminary consent. Samples within subjects across time were loaded in adjacent lanes. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes at 100 mA for 12 h. Primary antibodies of selected proteins of interest based on microarray results and gene network analyses included total signal transducer and activator of transcription (STAT)3, phospho-STAT3(Ser727), phospho-STAT3(Tyr705), total NF-κB p50, total IkBα, phospho-IκBα(Ser32/36), phospho-IκBα/β(Ser17/180), heat shock protein (HSP)27, and HSP70 (Cell Signaling Technologies, Beverly, MA). Appropriate primary antibody concentrations were determined in preliminary experiments and were 1:1,000 (vol/vol) horseradish peroxidase-conjugated secondary antibody was used at 1:50,000 (wt/vol), followed by chemiluminescent detection in a Bio-Rad ChemiDoc imaging system with band densitometry performed with Bio-Rad Quantity One (software package 4.5.1, Bio-Rad Laboratories, Hercules, CA). Parameters for image development were consistent across all membranes by predefined saturation criteria as described previously (3).

**Serum cytokines and creatine kinase.** Circulating concentrations of proinflammatory cytokines IL-6, IL-1β, IL-8, and TNF-α were determined by ELISA on 39 subjects [20 young (10 men, 10 women); 19 old (9 men, 10 women)] with MS2400 Human Pro-Inflammatory 4-Plex II Ultra-Sensitive Kits (Meso Scale Discovery, Gaithersburg, MD) and standard procedures. Samples were measured in triplicate. Serum creatine kinase (CK) activity was determined in 39 subjects (19 young (10 men, 9 women); 20 old (10 men, 10 women)) by a standard enzymatic rate method with the SYNCHRON DXc 800 system in the UAB Hospital Chemistry Laboratory. The baseline muscle biopsy was taken a minimum of 1 wk before the 24-h post-RL biopsy to prevent any residual effects of the baseline biopsy on circulating CK or cytokine levels.

**Data analysis.** Baseline differences between the older and younger adults in subject characteristics and protein expression were determined by independent t-tests. A 2 × 2 (age × RL) repeated-measures ANOVA was used to test age, time, and age × time interaction effects for serum cytokines, CK levels, and muscle protein expression/ phosphorylation with age and in response to RL (pre-RL vs. post-RL). Tukey’s honestly significant difference (HSD) tests were performed post hoc as appropriate. Significance was accepted at P < 0.05.
Microarray data analysis was performed as follows. Normalization of raw data and comparative analysis between expression profiles were carried out with Genespring GX 10.0 (Agilent Technologies). The raw data were normalized with quantile normalization and filtering of the transcripts by “present” and “marginal” flags. A “present,” “absent,” or “marginal” was designated to a transcript based on signal intensity and background noise. A transcript was kept if it had a “present” or “marginal” call for 100% of the arrays in at least one of the two treatment groups (pre-RL or post-RL). This reduced the number of transcripts from 41,078 to 25,597 in the older adults and to 25,337 in the younger adults. A paired t-test was used to determine RL-mediated differences in gene expression within each age group (old and young) at a stringent P value of <0.001 to minimize type I errors. To provide biologically meaningful information to the transcript profile changes, all differentially expressed transcripts were functionally annotated with the Database for Annotation, Visualization, and Integrative Discovery (DAVID, david.abcc.ncifcrf.gov) and Gene Ontology (GO, www.geneontology.org), and their interacting roles in networks, cellular functions, and canonical pathways were further analyzed with Ingenuity Pathways Analysis (IPA) 5.0 (Ingenuity Systems, Redwood City, CA).

RESULTS

There was a 46% increase in serum CK 24 h after RL in both the older and younger adults (P < 0.001, Table 1), indicating that the RL bout achieved the degree of modest damage to the quadriceps. Importantly, the degree of myofiber membrane wounding as indexed by serum CK was nearly identical in the two age groups. Additionally, post-RL there was an increase in circulating IL-6 in both the older and younger adults (P < 0.005, Table 1). There were no changes in circulating TNF-α or IL-8 levels (P > 0.05, Table 1). The multiplex plate that was used to determine changes in serum cytokine levels was not sensitive enough to sufficiently detect IL-1β in all samples.

Transcript profile results and functional annotation. Average fold changes of the genes found to be differentially expressed (P < 0.001) within each age group after RL are summarized in Supplemental Table S1. After unaccustomed RL, 351 genes were differentially expressed (128 downregulated and 223 upregulated) among the older adults. Of these, 318 were unique transcripts (for internal validation, the Agilent 4.1-fold). Under the broad classification of the transcripts by “present” and “marginal” flags. A “present,” “absent,” or “marginal” was designated to a transcript based on signal intensity and background noise. A transcript was kept if it had a “present” or “marginal” call for 100% of the arrays in at least one of the two treatment groups (pre-RL or post-RL). This reduced the number of transcripts from 41,078 to 25,597 in the older adults and to 25,337 in the younger adults. A paired t-test was used to determine RL-mediated differences in gene expression within each age group (old and young) at a stringent P value of <0.001 to minimize type I errors. To provide biologically meaningful information to the transcript profile changes, all differentially expressed transcripts were functionally annotated with the Database for Annotation, Visualization, and Integrative Discovery (DAVID, david.abcc.ncifcrf.gov) and Gene Ontology (GO, www.geneontology.org), and their interacting roles in networks, cellular functions, and canonical pathways were further analyzed with Ingenuity Pathways Analysis (IPA) 5.0 (Ingenuity Systems, Redwood City, CA).

Table 1. Subject characteristics and serum levels of creatine kinase and inflammatory cytokines before and 24 h after resistance loading

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Pre-RL</th>
<th>Post-RL</th>
<th>Pre-RL</th>
<th>Post-RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>73 ± 1*</td>
<td>37 ± 1</td>
<td>76.6 ± 2.6</td>
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<tr>
<td>Weight, kg</td>
<td>72.4 ± 2.1</td>
<td>67.9 ± 2.7</td>
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<td>LBM, kg</td>
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<td>50.35 ± 2.1</td>
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<td>Creatine kinase, U/l</td>
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<td>171.1 ± 31.5†</td>
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<tr>
<td>IL-6, pg/ml</td>
<td>1.44 ± 0.21</td>
<td>1.78 ± 0.25†</td>
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<tr>
<td>TNF-α, pg/ml</td>
<td>4.11 ± 0.63</td>
<td>4.21 ± 0.68</td>
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<tr>
<td>IL-8, pg/ml</td>
<td>10.88 ± 1.48</td>
<td>10.90 ± 1.33</td>
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Values are means ± SE for 22 and 24 (subject characteristics), 20 and 19 (creatine kinase levels) and 19 and 20 (IL-6, TNF-α, IL-8 levels) old and young subjects, respectively. RL, resistance loading; LBM, lean body mass. *Different from young, P < 0.05. †Different from baseline, P < 0.05.
49 \( (P = 10^{-49}) \) in which 29 of the 35 possible transcripts were differentially expressed after RL (Fig. 1 and Table 2). In agreement with the largest list of transcripts grouped via functional annotation, the two “hubs” of this network (NF-κB and p38 MAPK) gave a strong indication that inflammation and stress lay at the center of the primary skeletal muscle responses to unaccustomed RL among older adults. The translated protein products of transcripts within this network play key roles in stress and cellular compromise (HSPH1, HSPA9, HSP70, HSP27, HSPB1, SMARCA4, PLXNA1, NRP1); inflammation and immune responses (ZFP36, TRAF7, MAP2K3, MAP3K5, NRP1); necrosis (MAP3K5); protein synthesis (CTNNA1, JUP); protein degradation (TRAF7, ubiquitin, MAP2K3, MAP3K5); skeletal and muscular development (DYRK1B, MAP2K3, MYBPH, NRP1, SMARCA4, JUP, PLXNA1, NPTX1); cell growth and proliferation (TCP1, CCT5, FADS3, COL4A3); myoblast-myotube fusion and cell-cell adhesion (DYRK1B, CTNNA1, JUP); fibrosis and connective tissue function (PLXNA1, NRP1, COL4A3, MAP2K3); and structural integrity (MYBPH, SMARCA4, COL4A3, CTNNA1, JUP).

**Protein-level analysis.** Proteins were selected for follow-up immunoblotting based on microarray results that suggested stress- and cytokine-mediated inflammation among old subjects after RL. While no RL-mediated changes in protein content or phosphorylation state were found at the 24 h time point, some remarkable age differences in the resting muscle were identified and are described below. These age differences in protein expression at baseline suggest that the muscles of old adults are “primed” for a stress response and therefore may have a heightened sensitivity to cellular stress and inflammation.

IL-6 signaling is transduced largely via phosphorylation and activation of the transcription factor STAT3. While circulating IL-6 increased similarly (−25%) in old and young subjects after RL, STAT3 mRNA increased (1.4-fold) among old subjects only. We therefore quantified total and phosphorylated STAT3 protein in muscle lysate and found remarkably higher baseline levels of both total (+114%, \( P < 0.001 \)) (Fig. 2A) and phosphorylated (Tyr705 +96%, \( P < 0.005 \)) (Fig. 2B) STAT3 protein in old versus young subjects. Phosphorylation at Tyr705 induces STAT3 dimerization and nuclear localization.

Fig. 1. Ingenuity Pathways Analysis (IPA) Network 1 (score = 49) clustered 29 (of 35 in the network) of the resistance loading (RL)-mediated differentially expressed genes in older adults (\( n = 8 \)) that are directly and indirectly interacting and relevant to stress and cellular compromise (a); inflammation and immune responses (b); necrosis (c); protein synthesis (d); protein degradation (e); skeletal and muscular development (f); cell growth and proliferation (g); myoblast-myotube fusion and cell-cell adhesion (h); fibrosis and connective tissue function (i); and structural integrity (j). Red, upregulated; green, downregulated; white, not differentially expressed but related to this network. *Transcript represented by multiple probe sets. EG, Entrez Gene No. or GeneID. Two heat shock proteins (HSPs) inserted into the network by IPA, HSP27 and HSP70, are synonymous with the differently expressed HSPB1 and HSPA9, respectively. Such redundant entries occur by default in IPA’s automated networking function if a gene within, or synonymous with, a larger “group” enters the network (HSPA9 is synonymous with, or within, the HSP70 group, which contains 11 members; HSPB1 is synonymous with, or within, the HSP27 group, which contains 4 members).
Differentially expressed genes in older and younger adults, increased serum CK, and increased circulating IL-6 suggest that the unaccustomed high-intensity RL bout was sufficient to induce moderate damage in the skeletal muscle and initiate changes in the skeletal muscle transcriptome, particularly among older adults. We find it remarkable that, after the same mechanical stress, 318 genes were differentially expressed among old versus only 87 genes among young adults, with the two age groups showing similar changes in only two genes. These age differences in response to RL occurred despite similar degrees of myofiber membrane wounding as indexed by nearly identical percent increases in serum CK. The value of this finding should not be overlooked, as it indicates that the myofibers of older adult muscle were not more susceptible to mechanical membrane damage, yet they responded to the insult with a remarkably different gene expression profile that may help us begin to understand why regenerative function is impaired in the old as shown in rodent models (13, 14, 39).

Follow-up analyses centered on the a priori assumption that mechanical load-induced transcriptome changes unique to the old may underlie causes of age-related regeneration impairment, thus identifying attractive candidates for targeted studies. We therefore focused on the IPA network with the highest score (i.e., significance) among the old only. The two hubs of this network, NF-κB and p38 MAPK, suggest that inflammation and stress lay at the center of the primary skeletal muscle responses among the old.

**Repair and regeneration.** Skeletal muscle regeneration following injury appears dependent on the recruitment of resident SCs and/or other stem cell populations capable of differentiation along the myogenic lineage to fusion-competent myoblasts (15). These myoblasts then fuse as nuclear donors to damaged myofibers or, at sites of necrosis or severe damage, fuse to one another to form myotubes that differentiate into replacement fibers (10). We identified a few differentially expressed transcripts in the older adults 24 h after RL that may negatively impact SC-mediated processes during the subsequent recovery period (e.g., 48–96 h). For example, neuronal pentraxin 1 (NPTX1) and Dyrk1B/Mirk (minibrain-related kinase) were downregulated −2.4 and −1.4 fold, respectively. NPTX1 is transcriptionally activated by the myogenic transcription factor MyoD in proliferating myoblasts (39), while Dyrk1B is a Rho-induced kinase highly expressed in skeletal muscle that promotes myoblast fusion (18), and its expression normally increases coordinate with myogenin during myogenesis.Cell-cell adhesion is modulated by catenin Cα1 (CTNN1A1) and one of its binding partners, junction plakoglobin (JUP). Although CTNN1A1 increased slightly (1.2-fold), JUP was downregulated (−1.5-fold). Furthermore, calpastatin, a calpain inhibitor,
inhibits fusion and is normally downregulated during differentiation (6, 7) but was increased (1.3-fold). Collectively the differential expression of these genes at 24 h—among only the older adults—may negatively impact myoblast differentiation and fusion later during recovery when these processes are expected to be more prominent (e.g., 48–96 h).

Inflammatory and stress responses. We interpret several transcript-level alterations found only in the older adults to be suggestive of undue stress and inflammatory signaling within the muscle, which may be partially responsible for muscle regeneration impairment. Pavlath (43) showed quite clearly in mice that overt inflammation impairs regeneration, as IFN-γ-mediated muscle inflammation induced expression of class I major histocompatibility complex (MHC) in regenerating myofibers and attenuated regeneration. Analyzing a small number of target transcripts, two human studies indicate that aging alters the expression of pro- and anti-inflammatory genes in response to unaccustomed RL (20, 27). In the present study, the two main hubs within Network 1, NF-κB and p38 MAPK (Fig. 1), are intracellular intermediates of cytokine/stress signaling that are linked to numerous differentially expressed transcripts in Network 1. TNF-α and IL-1, two well-recognized proinflammatory cytokines with both systemic and local tissue effects, share common signaling pathways (NF-κB, SAPK/JNK, p38 MAPK). The upregulation after RL of TRAF7, a TNF receptor signal transducer and component of the ubiquitin ligase complex mediating protein degradation, along with higher levels of NF-κB protein in old versus young muscle despite no age differences in circulating TNF-α or IL-1, suggests heightened sensitivity in the older muscles to cytokine levels and/or cellular stresses. This concept is supported by recent evidence in rat primary SCs from old (vs. young) animals showing increased susceptibility to TNF-α induced proapoptotic signaling (36). Furthermore, in mice aging has been associated with greater stress-induced muscle expression of TNF-α, IL-1, and IL-6 (40). Muscle expression of TNF-α or IL-1 is also elevated in aging humans (25), and in aging rats increased TNF-α signaling (via NF-κB) induces apoptotic signaling, primarily in type II myofibers (47). Both TNF-α and IL-1 suppress protein synthesis in myoblasts and do so via a common second messenger, ceramide (53). Additional rodent data provide evidence that TNF-α promotes muscle protein degradation by increasing expression of the E3 ubiquitin ligase MuRF1 (1) and concomitantly impairs muscle protein synthesis by inhibiting mammalian target of rapamycin (mTOR)-mediated translation initiation (34). In the present study, PANX1, which regulates the release of IL-1 isoforms α and β (44), was robustly upregulated among old (2.9-fold) adults. Like TNF, IL-1 has been shown to induce muscle atrophy and inhibit protein synthesis, at least in part by blunting the expression of the e-subunit of eIF2B (eIF2Be) (16). Finally, MAP kinases in Network 1 that were upregulated include MAPK3 (MAP2K3; 1.7-fold) and MAPKKK5 (MAP3K5; 1.4-fold); both are intermediates in the transduction of several cytokine-mediated inflammatory and cell stress pathways.
responses including SAPK/JNK signaling, necrosis, and caspase-mediated apoptosis. The upregulation of MAP3K5 in muscle after unaccustomed RL persists for at least 48 h (38).

Increased expression of IL-6 is a common finding following mechanically induced muscle damage (55). There is some debate as to whether IL-6 signaling is beneficial or inhibitory during the acute response to mechanical stress. In mice, IL-6 has recently been shown to increase the expression of HSP72 and HSP25 in response to stress (29), suggesting an acute protective effect. On the other hand, chronic exposure to elevated muscle IL-6 induces atrophy (26), blunts growth (9), and is commonly considered a potential contributor to age-related sarcopenia. Congruent with the TNF/NF-κB results, our muscle protein-level analyses indicate much greater IL-6-associated signaling within muscle among older adults, despite no age differences in circulating IL-6. STAT3, the key transcription factor in IL-6 mediated JAK/STAT3 signaling as well as glucocorticoid receptor signaling, was upregulated 1.4-fold. Furthermore, a higher overall STAT3 protein level and state of STAT3 phosphorylation (Tyr705) among older adults suggests greater inflammatory signaling in the old even at rest. These findings again suggest that the older muscle may be more sensitive to any given amount of IL-6. Antimyogenic effects of IL-6 may be mediated at least partially by induced expression of the muscle E3 ubiquitin ligase atrogin-1/MAFbx and TNF-α (9). Recently in humans it has also been noted that acute mechanical stress robustly activates skeletal muscle STAT3 signaling (56), and, relevant to the present study, the investigators found that STAT3 phosphorylation was fivefold greater in old versus young while protein expression of the STAT3 antagonist suppressor of cytokine signaling (SOCS)-3 was repressed in the old (57).

Numerous members of the HSP family were upregulated among older adults as highlighted in Network 1. HSPs may play a role in degrading damaged proteins and maintaining cellular integrity after mechanical load-induced muscle injury. That we found higher HSP70 protein in the resting muscles of older adults and a greater HSP response to RL at the mRNA level suggests that the muscles of the old may be more actively remodeling to maintain integrity at rest, and may sense a greater need for HSP-mediated protection in response to the mechanical perturbation imposed by RL. Among young adults, the single HSP transcript that was upregulated (heat shock 70-kDa protein 1-like, HSPA1L) in the present study has been reported to be robustly induced in young men 3 h after a bout of 300 maximal eccentric contractions (38). Why this particular HSP transcript was unchanged in old subjects and appears to be consistently upregulated in young subjects after RL is unclear at this point. Overall, however, a far greater HSP response at the mRNA level was found among the old.

Five metallothionein (MT) isoforms (1A, 1B, 1G, 1H, and 1X) were downregulated (1.4- to 2.1-fold) in the older adults after RL. MTs play a protective role against oxidative stress, apparently as free radical scavengers, as heavy metal “buffers,” and/or by upregulating the expression of antioxidant enzymes (37). Five additional transcripts that function in metal ion binding were downregulated only among the old, including phosphodiesterase 11A (PDE11A), which was down 2.5-fold (see Supplemental Table S1). In prior studies of young men, seven MTs were upregulated after endurance exercise, presumably from the oxidative stress (37); however, when young men underwent a single bout of high-intensity eccentric RL, only one MT was differentially expressed and was downregulated (38). The oxidative nature of endurance exercise compared with RL suggests that the MTs may perhaps play different roles in stress management during recovery. Why MT expression was downregulated 24 h after RL among old subjects only is as yet unclear; however, this may be yet one more indication that the RL stimulus was much more of a stress in the old since the present literature suggests that a substantially higher degree of mechanical stress is required to elicit MT downregulation in the young (38).

**Protein metabolism.** Among older adults we found significantly increased mRNA expression of factors involved in translation, including eIF2B6 (1.3-fold), eIF4E (1.4-fold), and ribosomal protein S6 kinase-like 1 (RPS6KL1; 1.5-fold); on the other hand, expression of the upstream negative regulator—protein phosphatase 2A (PP2A)—was concurrently increased (1.4-fold). PP2A inhibits protein synthesis by dephosphorylating the mTOR targets 4EBP1 and p70S6K1, and, in fact, there is some evidence that mTOR’s kinase action on these two targets may not be direct but rather results from restraining PP2A via mTOR phosphorylation (inhibition) of PP2A (45). Additionally, PP2A may impair protein synthesis by directing ubiquitination of p70S6K1 (58). AMP-activated protein kinase (AMPK) signaling is also known to counteract Akt/mTOR signaling, and the γ-2 subunit of AMPK (PRKAG2) increased 1.5-fold among the old. The chaperone HSP27, also upregulated among the old, inhibits protein synthesis by binding eIF4G and thus preventing assembly of the eIF4F cap-binding initiation complex (17). Among older subjects, several components of the Ub-proteasome system were differentially expressed after RL, including the peptidases USP28 (+1.5-fold) and USP4 (-1.3-fold), an E2 conjugating enzyme (UBE2G1; -1.4-fold), and UBF1 (-1.2-fold). At this point the nature of these transcript-level changes in the translational machinery and Ub-proteasome system among only the older subjects is unclear, yet these data provide further evidence that the RL bout was interpreted by the muscles of old subjects as a much more potent stimulus of transcriptional activity.

**Structural integrity of skeletal muscle.** Some noteworthy genes that were differentially expressed only in older subjects after RL support the concept that the muscles of older subjects may have experienced a degree of stress far exceeding that in young subjects despite being exposed to the exact same stressor. For example, gene expression of MyBPH was robustly elevated (+1.5-fold) and USP4 (-1.3-fold), an E2 conjugating enzyme (UBE2G1; -1.4-fold), and UBF1 (-1.2-fold). At this point the nature of these transcript-level changes in the translational machinery and Ub-proteasome system among only the older subjects is unclear, yet these data provide further evidence that the RL bout was interpreted by the muscles of old subjects as a much more potent stimulus of transcriptional activity.

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sion is modulated by the transcription factor SMARCA4 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4), which was also significantly upregulated in the old only. Interestingly, SMARCA4 is activated by glucocorticoid receptor signaling and, in turn, regulates the expression of notable muscle-specific genes including myogenin, troponin T, and MyBP. A strain on muscle integrity among the old was also suggested by significant downregulation (~1.7-fold) of both type IV collagen α3 (COL4A3) and α4 (COL4A4) mRNA expression and 1.6-fold upregulation of TUBA8. Type IV collagen, a major constituent of basement membranes, is degraded by matrix metalloproteinases (MMP-2 and MMP-9) in response to muscle damage (49). These findings suggest that the muscles of the older subjects may have been attempting to launch a compensatory effort to maintain structural integrity—a response to this degree was apparently not sensed as necessary among the younger subjects.

Limitations. While we feel that the results revealed from this study reveal valuable information on postexercise inflammatory and regenerative responses in older adults, there are limitations. We recognize that changes in the skeletal muscle transcript profile in response to exercise are transient (38) and that the single time point of 24 h after RL limits our ability to detect early, more transient changes. Differential responses between older and younger adults could therefore be partially due to timing; however, previous transcript profiling studies in younger adults do not demonstrate such marked stress and inflammatory responses even at earlier time points after RL (38, 60). Microarray analysis of RNA isolated from tissues of mixed cellularity such as skeletal muscle is complicated by the loss of cellular and spatial information defining the signal origin (22). While the bulk of RNA isolated from muscle tissue is derived from myonuclei, age differences in the abundance of other contributing cell types could be an important factor that was not controlled. This possible confounder, however, should have been minimized since our analysis focused on within-subject changes in response to RL (rather than cross-sectional age group comparisons). Microarray technology itself is not without inherent limitations, including background noise and the sensitivity and specificity of probes (22). We took great care to control for these issues by employing quantile normalization and by filtering (flagging) the transcripts based on signal intensity and background noise. Finally, the number of samples/subjects analyzed via microarray did not allow us to statistically test interactions between age and RL (rather than cross-sectional age group comparisons). Microarray technology itself is not without inherent limitations, including background noise and the sensitivity and specificity of probes (22).

Summary. We have described, for the first time, marked age differences in the genomewide transcriptome response to a standardized, unaccustomed bout of RL. Together, the numerous age differences noted strongly suggest that the threshold of mechanical stress required to induce changes in the molecular signature of skeletal muscle is much lower in the old. The muscles of old (vs. young) adults were much more sensitive to an equal and modest degree of damage—launching a robust transcriptome-level response that may begin to reveal key differences in the regenerative capacity of skeletal muscle with advancing age. With nearly fourfold more genes differentially expressed in old versus young adults in response to RL, we found the muscle transcriptome of the old to be responsive across a wide array of functional gene groupings including inflammation and stress, repair, structural integrity, protein metabolism, apoptosis, and proliferation among others. These novel data provide an important basis for future investigations aimed to target age differences in specific cellular processes that may help us to better understand the impact of aging on muscle regenerative function.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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