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RESEARCH ARTICLE

Transcriptome response of human skeletal muscle to divergent exercise stimuli

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Dickinson JM, D'Lugos AC, Naymik MA, Siniard AL, Wolfe AJ, Curtis DP, Huentelman MJ, Carroll CC. Transcriptome response of human skeletal muscle to divergent exercise stimuli. *J Appl Physiol* 124: 1529–1540, 2018. First published March 15, 2018; doi:10.1152/japplphysiol.00014.2018.—Aerobic (AE) and resistance exercise (RE) elicit unique adaptations in skeletal muscle that have distinct implications for health and performance. The purpose of this study was to identify the unique transcriptome response of skeletal muscle to acute AE and RE. In a counterbalanced, crossover design, six healthy, recreationally active young men (27 ± 3 yr) completed acute AE (40 min of cycling, ~70% maximal HR) and RE [8 sets, 10 reps, ~65% 1-repetition maximum (1RM)], separated by ~1 wk. Muscle biopsies (vastus lateralis) were obtained before and at 1 and 4 h postexercise. Whole transcriptome RNA sequencing (HiSeq2500; Illumina) was performed on cDNA synthesized from skeletal muscle RNA. Sequencing data were analyzed using HTSeq, and differential gene expression was identified using DESeq2 [adjusted *P* value (FDR) <0.05, >1.5-fold change from preexercise]. RE resulted in a greater number of differentially expressed genes at 1 (67 vs. 48) and 4 h (523 vs. 221) compared with AE. We identified 348 genes that were differentially expressed only following RE, whereas 48 genes were differentially expressed only following AE. Gene clustering indicated that AE targeted functions related to zinc interaction, angiogenesis, and ubiquitination, whereas RE targeted functions related to transcription regulation, cytokine activity, cell adhesion, kinase activity, and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. *ESRRG* and *TNFRSF12A* were identified as potential targets related to the specific response of skeletal muscle to AE and RE, respectively. These data describe the early postexercise transcriptome response of skeletal muscle to acute AE and RE and further highlight that different forms of exercise stimulate unique molecular activity in skeletal muscle.

NEW & NOTEWORTHY Whole transcriptome RNA sequencing was used to determine the early postexercise transcriptome response of skeletal muscle to acute aerobic (AE) and resistance exercise (RE) in untrained individuals. Although a number of shared genes were stimulated following both AE and RE, several genes were uniquely responsive to each exercise mode. These findings support the need for future research focused to better identify the role of exercise mode as it relates to targeting specific cellular skeletal muscle abnormalities.

aerobic; estrogen-related receptor-γ; fibroblast growth factor receptor-14; resistance; RNA sequencing

INTRODUCTION

Skeletal muscle is a highly adaptable tissue capable of responding to alterations in functional demands through a variety of internal and external stimuli. Exercise in particular presents a unique stimulus to skeletal muscle and is known to facilitate changes to the contractile, structural, and metabolic properties within the muscle fibers as well as to the extracellular environment (e.g., capillaries, connective tissue). The specific adaptive responses within skeletal muscle are dependent in part on the type of exercise performed. Specifically, it is well understood that resistance exercise (RE) training generally produces increases in muscle size and strength through the accumulation of contractile proteins (5, 31), whereas aerobic exercise (AE) training improves oxidative capacity and fatigue resistance through the accumulation of mitochondrial proteins (23, 55). However, the precise mechanisms through which divergent exercise stimuli elicit their unique skeletal muscle adaptations remain to be completely understood.

Ever since the seminal work by Holloszy (34), Gollnick et al. (30), and others (see Ref. 33) highlighting the unique cellular responses of skeletal muscle to AE, our understanding of the molecular mechanisms regulating adaptations of skeletal muscle to various forms of exercise has grown exponentially. Whereas countless studies have identified several mechanisms regulating the response of skeletal muscle to a single form of exercise, less attention has been given to identify those mechanisms that may be responsible for the distinct skeletal muscle adaptations that are facilitated by different forms of exercise (i.e., AE vs. RE). Furthermore, those studies that have compared the postexercise cellular response of skeletal muscle between AE and RE to date have focused largely on examination of specific signaling pathways, genes, proteins, or molecular processes (12, 13, 42, 57, 64, 78, 80). Although these studies have uncovered intriguing exercise mode-specific responses, there still remains a need to compare the adaptive response of skeletal muscle between AE and RE using a more expansive approach. Specifically, emerging technology, such as whole transcriptome RNA sequencing (RNA-seq), has improved the ability to more comprehensively examine wide-ranging molecular mechanisms in a manner devoid of selection

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bias. Although studies have employed microarray methods to investigate the skeletal muscle transcriptome response to exercise (44, 60, 65, 74, 75), to our knowledge, limited data are available in which whole transcriptome RNA-seq has been used to examine the transcriptome response of human skeletal muscle, particularly to divergent acute exercise modes. Recently, Robinson et al. (66) employed RNA-seq and demonstrated that different forms of exercise produce unique changes in the basal transcriptome of skeletal muscle. However, it is important to note that the adaptive properties of skeletal muscle are also dependent upon the repeated stimulation of molecular events in response to each acute exercise bout, and particular focus should be given to the complex transcriptional response to each exercise stimulus (26). In addition, the acute transcriptional response to exercise, even in the trained state, may be of greater magnitude compared with changes observed in the basal condition in response to training (65). Thus, examination of the acute transcriptional response of skeletal muscle to divergent exercise stimuli is likely to provide its own unique perspective on the specific adaptation of skeletal muscle to different forms of exercise as well as provide a means to identify molecular targets of AE and RE (26).

Enhancing the ability for exercise to preserve and improve health, and the need to identify the specific mechanisms involved, has been highlighted recently (6, 58). Importantly, AE and RE elicit unique molecular changes (71) and adaptations in skeletal muscle that have overlapping as well as distinct implications for both health and performance. Specifically, to test our hypothesis that divergent exercise stimuli elicit unique transcriptional activity in human skeletal muscle, the purpose of this study was to utilize whole transcriptome RNA-seq to determine the early postexercise (1 and 4 h) transcriptome response of skeletal muscle to acute AE and RE in otherwise untrained individuals.

MATERIALS AND METHODS

Participants

Seven healthy men from the greater Phoenix, AZ, metropolitan area volunteered to participate in this study. All participants were considered recreationally active but not engaged in a regularly scheduled AE or RE training program (>2 days/wk). Screening was performed with medical history and physical activity questionnaires as well as a blood draw for coagulation profile. All participants provided written, informed consent before participation. For reasons unrelated to study procedures, one participant dropped out of the study before completing an experimental trial, and thus data are presented on the six participants that completed the study (means ± SD: 27 ± 3 yr, 179 ± 6 cm, 79 ± 10 kg). All procedures were approved by the Institutional Review Board of Midwestern University (in compliance with the Declaration of Helsinki, as revised in 1983).

Prior to the experimental trials (see below), maximal unilateral knee extension strength [1-repetition maximum (1RM)] and maximal cycling exercise heart rate and peak workload (W) were determined for each participant. Specifically, 1RM was determined unilaterally for each leg on two separate occasions using a knee extension device (Cybex VR3, Medway, MA) (59). The first 1RM measurement was collected during the initial screening and the second ~1 wk before study participation. The heaviest weight lifted by each leg between the two measurements was considered the participant's 1RM for each leg. Maximal exercise heart rate and peak workload were determined on a stationary cycle (model 828E; Monark Exercise, Vansboro, Sweden) using a ramp protocol. Participants began cycling at low workload (~50 W), and then the workload was progressively increased by 15 W every minute until volitional fatigue. During the test, participants' heart rate was measured via telemetry, and the maximal heart rate and peak workload (W) were recorded. For descriptive purposes, $\dot{V}O_{2peak}$ for each participant was estimated using peak cycling workload and standard metabolic equations for leg cycling: $\dot{V}O_2$ (ml·kg^{-1}·min^{-1}) = 1.8 × (work rate in kg·m^{-1}·min^{-1})/(body mass in kg) + 3.5 ml·kg^{-1}·min^{-1} + 3.5 ml·kg^{-1}·min^{-1} (29).

Study Design

Participants were studied during two separate experimental trials (Fig. 1). Trials were separated by ~1 wk and were performed using a randomized, counterbalanced crossover design. Both experimental trials were identical except for the mode of exercise performed (aerobic or resistance). Subjects were also instructed to abstain from alcohol consumption for 72 h before each experimental trial and from caffeine for 24 h before each experimental trial.

Experimental trials. On the morning of each experimental trial (Fig. 1), participants arrived at the laboratory (~0700) following a 12-h overnight fast and remained fasted until completion of the trial (but were provided water ad libitum). Participants were asked to record their evening meal before the first experimental trial and to repeat the same evening meal before the second experimental trial. During the first trial, a basal muscle biopsy was obtained following 30 min of supine rest. To minimize total muscle biopsies performed on each participant, the basal biopsy from the first trial was used as the basal sample for both experimental trials, and therefore, no basal muscle biopsy was obtained during the second experimental trial. After the basal biopsy (1st trial) or 45 min of supine rest (2nd trial, to account for the time needed for all procedures related to the muscle biopsy during the first trial), participants performed a 5-min warmup at a low workload on a cycle ergometer (model 828E; Monark Exercise) and then performed either the acute AE bout or the acute RE bout. Following exercise, participants rested supine on a clinical bed, engaging in minimal physical activity for the remainder of the trial. Muscle biopsies were obtained at 1 and 4 h postexercise. Following a washout period (means ± SD: 9 ± 3 days), the second experimental trial was repeated in which the participant performed the other exercise mode. For the six participants that completed the experimental trials, three participants performed AE during their first experimental trial, and three participants performed RE during their first experimental trial. In addition, the use of the dominant leg for the basal biopsy and for the postexercise biopsies for each exercise mode was also counterbalanced. Importantly, the counterbalanced crossover design minimizes the influence of factors unrelated to the exercise

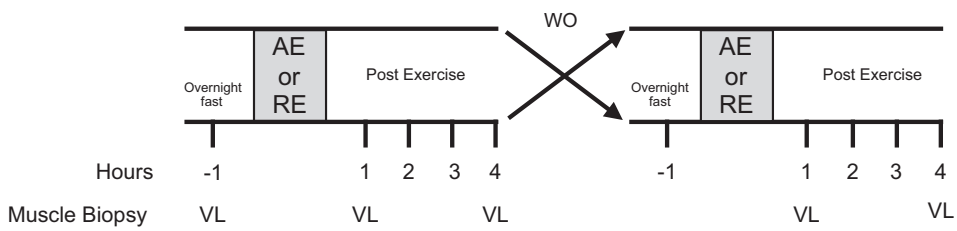

Fig. 1. Experimental trials schematic. Participants were studied during 2 separate experimental trials that were performed using a randomized, counterbalanced crossover design. Each experimental trial was performed after an overnight fast. Trials were separated by 9 days on average. AE, aerobic exercise; RE, resistance exercise; VL, vastus lateralis muscle biopsy; WO, washout.

stimuli (biopsy procedure, time of day), particularly with regard to identifying the unique transcriptome response to each exercise mode.

Acute exercise bouts. The exercise bouts consisted of an acute session of either AE or RE. AE consisted of 40 minutes of stationary cycling (model 828E; Monark Exercise, Vansboro, Sweden) at a prescribed initial intensity corresponding to ~70% maximal heart rate. Heart rate was continuously monitored (Polar Electro, Lake Success, NY) throughout the exercise bout. Workload, heart rate, and rating of perceived exertion were collected every 2 min during the exercise bout. RE consisted of isotonic unilateral leg extensions (Cybex VR3), as we have conducted previously (16, 59), in which each leg completed eight sets of 10 repetitions at ~60–65% of the 1RM for that leg. Both legs were exercised to mimic the bilateral nature of cycling; however, unilateral exercise was chosen to ensure that the leg to be biopsied postexercise performed equal work (e.g., 3 of the participants had the dominant leg biopsied post-RE, and 3 of the participants had the nondominant leg biopsied post-RE). Participants alternated legs such that the leg to be biopsied performed each set first (e.g., 1 leg performed 10 reps and then the other leg performed 10 reps, repeated 8 times). Participants were provided with 3 min of rest between sets. Total exercise time for the RE bout was ~40 min.

Muscle biopsies. Muscle biopsy procedures were conducted under strict sterile conditions. All muscle biopsies were obtained from the lateral portion of the vastus lateralis following local anesthesia (1% lidocaine without epinephrine) using a 5-mm Bergström biopsy needle with suction (9). The basal muscle biopsy was obtained from the nondominant leg only during the first experimental trial, which served as basal for both exercise trials (see above). During the experimental trials, the 4-h postexercise biopsy was obtained from the same incision as the 1-h postexercise biopsy; however, the biopsy needle was inclined at a different angle such that the 4-h postexercise biopsy was taken ~5 cm proximal to the 1-h postexercise biopsy, as previously performed (16–19, 59, 77). All muscle samples were obtained from a single pass of the needle at each time point. The 1- and 4-h biopsies during the first experimental trial were obtained from the opposite leg as the basal biopsy, whereas the 1- and 4-h biopsy during the second trail were obtained from the same leg used for the basal biopsy during the first experimental trial. Muscle tissue was immediately blotted, dissected of connective and adipose tissue, and frozen in liquid nitrogen. Muscle tissue was stored at −80°C until analysis.

Analytical Techniques

RNA isolation. Total RNA was isolated from frozen muscle tissue (mean ± SD: 28.4 ± 6.8 mg) using a handheld homogenizer (BioGen PRO200; Pro Scientific, Oxford, CT) in a solution containing 1.0 ml of TRI Reagent (Molecular Research Center, Cincinnati, OH), as previously described (19, 59). The RNA was separated into an aqueous phase using 0.20 ml of chloroform and precipitated using 0.50 ml of isopropanol. The RNA pellet was then washed with 1.0 ml of 75% ethanol, dried, and dissolved in a predetermined amount (1.5 μl/mg tissue) of nuclease free water. RNA concentration was assessed in duplicate using a Take3 plate and Synergy H1 microplate reader (BioTek, Winooski, VT). To ensure genomic DNA was removed, 5 μg of RNA was treated with DNase I using a commercially available kit (DNA-free Kit; Ambion, Austin, TX). The mean ± SD 260/280 ratio of the resulting RNA was 1.937 ± 0.026. Integrity of the RNA was assessed on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The mean ± SD RNA integrity number (RIN) of the RNA was 8.84 ± 0.53.

Transcriptome sequencing. DNAse-treated RNA was quantified with RiboGreen (Thermo Fisher Scientific, Waltham, MA). Four nanograms of RNA was used for cDNA synthesis, following the standard operating procedures of the laboratory, and the cDNA was prepared with Clontech's SMARTer Universal Low Input RNA kit (Takara Bio USA, Mountain View, CA). Libraries were prepared using the Clontech Low Input Library Prep kit version 2, and the resulting cDNA was checked for quality using D1000 high-sensitivity screen tape on a TapeStation 2200 (Agilent Technologies, Santa Clara, CA). All samples were barcoded, and RNA-seq was performed on an Illumina HiSeq 2500 instrument using 75-bp paired-end reads (Illumina, San Diego, CA). Average sequencing depth across samples was 24.3 million reads.

Quality control. Read quality was checked as described previously (61) using FastQC (3), which analyzes base and sequence quality, GC content, sequence lengths, duplication, and adapter content. Alignment quality was verified using Picard (Broad Institute, Cambridge, MA) for various sequence alignment metrics including alignment percent, insert size, and the distribution of genomic features. Principal component analysis was used to identify any outlier samples using a cutoff of 1.5× outside the interquartile range (no samples were determined as outliers). The sequencing data presented in this article have been submitted to the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) and are accessible through GEO Series accession no. GSE107934.

Bioinformatic analyses. Sequenced reads contained in the FASTQ files were aligned to the GRCh37 reference genome using STAR (20). Specifically, Binary Alignment Map files were used to build a read count table at the gene level using HTSeq (2). One sample from AE at 4 h failed library preparation and was not included in the analyses.

Differentially expressed genes from basal were identified independently for all postexercise time points using the R package DESeq2 (43). The statistical method implemented in DESeq2 fits a generalized linear model for each gene, with modeling read counts following a negative binomial distribution. The logarithmic fold change, expressed as log2 fold change, was estimated with an Empirical Bayes procedure, whereas the significance was assessed with a Wald test. The *P* values were corrected with the Benjamini-Hochberg method (7), and genes were annotated using the R-Biomart package (21), retrieving Ensemble ID and HGNC gene symbols according the GRCh37.p13 assembly. Genes with an adjusted *P* value (FDR) of ≤ 0.05 and absolute log2 fold change of >0.58 or less than −0.58 (1.5-fold) was considered differently expressed from basal. UpSet plots were constructed as described by Conway et al. (14).

Enrichment analysis was performed by the means of DAVID web tool (35, 36), using as input the differentially expressed gene lists obtained for each exercise mode and postexercise time point and the hypergeometric statistics with the probability density method function. Enrichment for all gene lists was computed using the information from the KEGG (38), Reactome (15, 53), Panther (51, 52), GO (4), Interpro (24), PFAM (25), SMART (67), COG (72), and Uniprot (76) databases updated to October 2016. All *P* values obtained from the enrichment analysis were corrected using the Benjamini-Hochberg method (7). The gene ontology biological process and KEGG pathway annotations from DAVID were visualized with the Cytoscape (69) plugin enrichment map (48) to display annotations between and within each exercise mode. Only the significant gene lists from the 4 h AE and 4 h RE time points were used, and only annotations with adjusted (Benjamini-Hochberg) *P* values < 0.05 were included. DAVID further clusters the enrichment annotation results using a fuzzy heuristic clustering algorithm based on the hypothesis that similar annotations should have similar gene members. Each cluster is assigned an EASE score, which is derived from the geometric mean of the individual annotation *P* value. Clusters from the DAVID web tool were derived using a classification stringency of Medium.

RESULTS

Physiological Exercise Data

Maximal heart rate and peak workload (means ± SD) achieved during the stationary cycling ramp protocol were 184 ± 14 beats/min and 225 ± 41 W, respectively. The esti-

Table 1. *Physiological data from the acute aerobic and resistance exercise bouts*

Variable	Average During Exercise	Peak Value[b]
Aerobic exercise[a]		
Heart rate, beats/min	147 ± 10 (138–166)	163 ± 15 (145–182)
$\%HR_{max}$	79 ± 4 (75–84)	89 ± 6 (82–96)
Work (W)	115 ± 28 (68–150)	130 ± 26 (90–165)
%Peak work	51 ± 5 (46–59)	58 ± 4 (53–65)
RPE	15 ± 1 (14–16)	17 ± 1 (16–18)
Resistance exercise		
Absolute weight lifted, kg	38 ± 12 (25–59)	44 ± 11 (30–61)
%1RM	61 ± 6 (52–68)	68 ± 7 (55–72)

Data are means ± SD (range). HRmax, maximal heart rate; RPE, rating of perceived exertion; 1RM, 1-repetition maximum. [a]Presented data are from *minutes 6* to *40* of exercise to allow for presentation of steady-state exercise; [b]For resistance exercise, peak exercise set; for aerobic exercise, peak 2-min average data from *minutes 6* to *40*.

mated $\dot{V}O_{2peak}$ for the participants was 38 ± 4 $ml \cdot kg^{-1} \cdot min^{-1}$. The participants' unilateral knee extension 1RM was 65 ± 17 kg. Descriptive exercise data during the AE and RE experimental trials are presented in Table 1. Mean power output during the AE bout (*minutes 6–40*) was ~51% peak power (~115 W). The highest 2-min average mean power during the AE bout was ~58% peak power (~130 W). The mean %1RM for all sets during the RE bout was ~61%, whereas the highest mean %1RM for a given set during the RE bout was ~68% 1RM.

Total Number of Differentially Expressed Genes

Differentially expressed genes for each exercise mode and post exercise time point are listed in Supplemental Table S1 (Supplemental Material for this article can be found on the *Journal of Applied Physiology* website). Both AE and RE stimulated a greater number of differentially expressed genes at 4 h postexercise relative to 1 h postexercise (Fig. 2 and Table 2). Furthermore, a majority of the differentially expressed genes following each exercise mode increased from basal, whereas relatively few differentially expressed genes were reduced in their expression from basal (Fig. 2 and Table 2). In addition, there were no discordant directional changes in gene expression observed between exercise modes at either postexercise time point [i.e., no genes that changed in the opposite direction (up vs. down) between exercise modes were identified]. However, relative to the response to AE, RE stimulated a change in expression of ~40% more genes at 1 h postexercise (AE: 48; RE: 67) and ~137% more genes at 4 h postexercise (AE: 221; RE: 523).

Exercise Mode-Specific Genes

Of the total genes differentially expressed in response to AE, 48 of these genes were only responsive to AE (not differentially expressed in response to RE at either postexercise time point; Fig. 2 and Supplemental Table S2). Five of these genes were differentially expressed at 1 h postexercise (4 increased, 1 decreased), 42 of these genes were differentially expressed at 4 h postexercise (21 increased; 21 decreased), and one gene (*RP11-876N24.4*; no known function) was differentially expressed (increased) at both 1 and 4 h (Table 3). Table 4 displays the top 10 most significant (based on adjusted *P* value) genes differentially expressed only in response to AE (4 h postexercise). Of these 10 genes, two are associated with ubiquitination (*RHOBTB2*; *TRIM68*) and two are associated with fiber type transitions to myosin heavy chain (MHC) I fibers (*ESRRG*; *TMEM140*).

Of the total genes differentially expressed in response to RE, 348 of these genes were only responsive to RE (not differentially expressed in response to AE at either post exercise time point) (Fig. 2 and Supplemental Table S2). Four of these genes were differentially expressed at 1 h postexercise (3 increased, 1 decreased), 334 of these genes were differentially expressed at 4 h postexercise (260 increased; 74 decreased), and 10 genes were differentially expressed at both 1 and 4 h (all increased) (Table 3). Furthermore, of these 10 genes differentially expressed at both 1 and 4 h post-RE, four are associated with progression through the cell cycle (*USP2*, *FOS*, *IRF2BP2*, *VGLL2*, and *DUSP1*), three are associated with the heat shock protein response (*HSPB8*, *DNAJA4*, and *IER5*), one is associated with actin polymerization (*LMOD2*), and one is of unknown function (*RP11-309L24.2*) (Table 3). Table 5 displays the top 10 most significant (based on adjusted *P* value) genes differentially expressed only in response to RE (4 h postexercise). Of these 10 genes, four are associated with structural integrity (*MYO18B*, *ITIH6*, *FLNC*, *KLHL40*), and three are associated with the heat shock protein response (*DNAJB5*, *DNAJA4*, *HSPB8*).

Figure 3 displays the transcriptional response for two select targets, *ESRRG* and *TNFRSF12A*. Specifically, *ESRRG* was identified as the third-most significant gene differentially expressed only following AE (Table 4), and *TNFRSF12A* was identified as the fifth-most significant gene differentially expressed only following RE (Table 5).

Exercise Mode-Specific Functional Categories (Gene Clusters) and Pathways

Functional categories based on gene clusters (see Supplemental Table S3) for each exercise mode and postexercise time point were derived from DAVID analyses using the exercise- and time point-specific list of differentially expressed genes. Using an a priori cutoff for enrichment score of 1.95, four enriched clusters were identified at 1 h post-AE, and five enriched clusters were identified at 1 h post-RE. At 1 h postexercise, both exercise modes stimulated gene clusters with terms related to angiogenesis functions (author-defined). Specific to each exercise mode, AE uniquely stimulated genes enriched with author-defined cellular signaling functions (terms including insulin resistance, AMPK signaling pathway, FoxO signaling pathway, insulin-signaling pathway, and adipocytokine signaling pathway), whereas RE uniquely stimu-

Fig. 2. UpSet plot (14) for the total no. of differentially expressed genes from basal (*A*), differentially expressed genes that were increased (upregulated) from basal (*B*), and differentially expressed genes that were decreased (downregulated) from basal in skeletal muscle at 1 and 4 h post-aerobic (AE) and -resistance exercise (RE) (*C*). Bars represent no. of differentially expressed genes shared by condition and postexercise time point that are identified by linked dots below the *x*-axis. Horizontal columns represent total no. of differentially expressed genes for each exercise mode and postexercise time point.

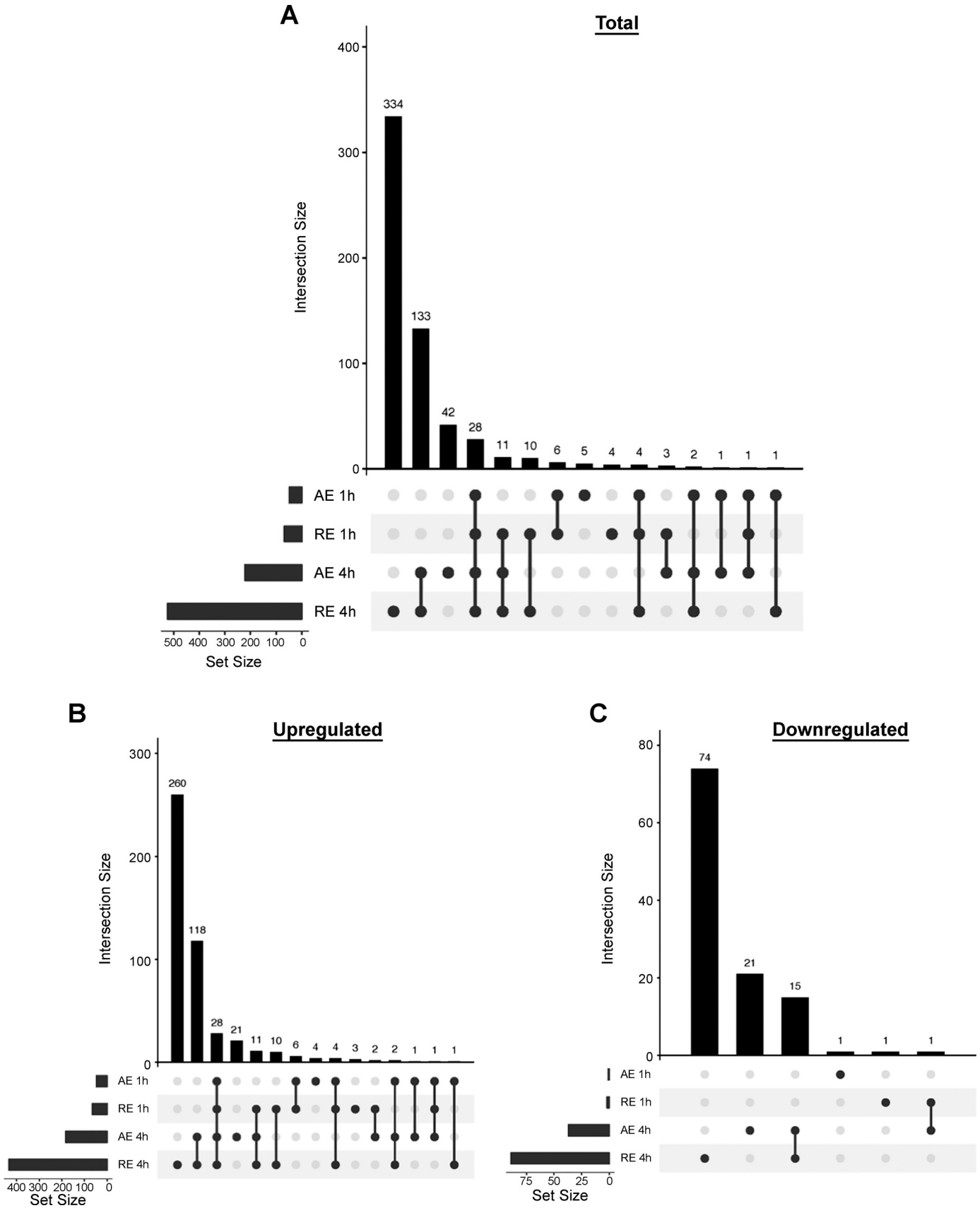

Table 2. *Total detected genes, significantly differentially expressed genes, and differentially expressed genes with ≥1.5-fold change from baseline*

Condition	Total Detected[a]	Significant[b]	1.5-Fold Change[c]	Directional Change[d]	
				Up	Down
Baseline	10,870				
Aerobic					
1 h	10,335	48 (0.5%)	48	47 (98%)	1 (2%)
4 h	11,040	222 (2%)	221	184 (83%)	37 (17%)
Resistance					
1 h	10,816	67 (0.6%)	67	65 (97%)	2 (3%)
4 h	10,343	534 (5%)	523	434 (83%)	89 (17%)

[a]Total no. of genes ≥5 counts; [b]no. of differentially expressed genes with adjusted (Benjamini-Hochberg) P value of ≤ 0.05 vs. baseline and in parentheses is %total detected genes; [c]no. of genes with adjusted (Benjamini-Hochberg) P value of ≤ 0.05 vs. baseline and ≥1.5-fold change from baseline; [d]no. of genes that were increased (up) or decreased (down) from baseline and in parentheses is the percentage of those that changed by 1.5-fold.

lated genes enriched with author-defined transcription regulation functions (see Supplemental Table S3 for terms within clusters).

At 4 h postexercise, nine enriched clusters were identified in response to AE, whereas 11 enriched clusters were identified in response to RE. Specific to each exercise mode, AE uniquely stimulated genes enriched with author-defined functions included zinc interaction, angiogenesis, and ubiquitination functions (see Supplemental Table S3 for terms within clusters). RE uniquely stimulated genes enriched with author-defined functions included transcription regulation, cytokine activity, cell adhesion, and kinase activity (see Supplemental Table S3 for terms within clusters).

KEGG pathway analyses were used to identify shared and unique pathways responsive to AE and RE. We focused on the 4-h postexercise time point given the greater number of differentially expressed genes (vs. 1 h). A change in the expression of genes associated with the TNF signaling pathway was observed following both AE and RE (Supplemental Fig. S5). No uniquely enriched pathways were identified from the differentially expressed genes identified 4 h post-AE. However, the phosphatidylinositol 3-kinase (PI3K)-Akt pathway was uniquely enriched following RE (Supplemental Fig. S5), including 24 genes associated with the pathway highlighted in Supplemental Table S4. Shared and unique KEGG pathway and gene ontology biological process annotations determined by DAVID at 4 h post exercise for each exercise mode are presented in Supplemental Fig. S5.

DISCUSSION

The goal of this study was to provide a foundation from which to begin to identify potential molecular targets of AE and RE as well as the potential mechanisms through with AE and RE may elicit their unique skeletal muscle adaptations. To accomplish this goal, we specifically employed whole transcriptome RNA-seq to identify the transcriptome response of skeletal muscle to acute AE and RE. In particular, relative to targeted approaches (e.g., PCR) and microarray methods, RNA-seq presents a broader dynamic range (especially for genes that are expressed at low levels), an increased specificity due to the use of sequence-based mapping that is important for the quantitation of gene family members that may have high homology, and an ability to identify novel genes that may not be represented on the prespecified content of a microarray. Although we identified a number of shared genes, biological processes, and gene clusters that were stimulated following both AE and RE, several genes that were uniquely responsive to each exercise mode were also identified. The results from this study highlight the distinct immediate whole transcriptome and biological response of skeletal muscle to divergent forms of acute exercise and further

Table 3. *Exercise mode specific genes differentially expressed at 1 h and 4 h post exercise*

Ensembl ID	Gene	Adjusted P Value		Description[a]	Ref. No(s).
		1 h	4 h		
Aerobic exercise					
ENSG00000262222	*RP11-876N24.4*	0.000074	0.000060	Unknown function	
Resistance exercise					
ENSG00000036672	*USP2*	0.005001	0.000066	Deubiquitinase promotes muscle cell differentiation, positive regulator of NF-κB signaling	50, 79
ENSG00000170345	*FOS*	0.022154	0.001445	Promotes proliferation and differentiation	37
ENSG00000168264	*IRF2BP2*	0.007237	0.010600	Transcription factors/coactivator of VEGFA; promotes regeneration	73
ENSG00000170162	*VGLL2*	0.003658	0.000090	Cofactor with TEF-1 and MEF2; promotes differentiation and myogenesis	45
ENSG00000120129	*DUSP1*	0.001122	0.045017	Interacts with MyoD; coordinates proliferation, differentiation, and repair	70
ENSG00000152137	*HSPB8*	0.032055	0.000002	Chaperone that regulates filamin breakdown; phosphorylated by PKC and ERK1	8
ENSG00000140403	*DNAJA4*	0.010726	0.000002	Heat shock protein	
ENSG00000162783	*IER5*	0.002861	0.000386	Regulator of cell proliferation; promotes chaperone gene expression, indirectly regulates S6K1 phosphorylation	39
ENSG00000170807	*LMOD2*	0.024249	0.003467	Regulated thin filament assembly and structure	11
ENSG00000242902	*RP11-309L24.2*	0.044619	0.000002	Unknown function	

ERK1, mitogen-activated protein kinase 3; MEF2, myocyte enhancer factor-2; NF-κB, nuclear factor κ-light-chain enhancer of activated B cells; PKC, protein kinase C; S6K1, ribosomal protein S6 kinase 1; TEF, transcription enhancer factor; TF, transcription factor; VEGFA, vascular endothelial growth factor A. [a]Descriptions obtained via UniProt (uniprot.org) and specific references when listed.

Table 4. *Top 10 differentially expressed genes (by adjusted P value) responsive only to AE (not identified as significantly differentially expressed in response to RE at 1 or 4 h; for comparative purposes, the fold change of these genes to RE is also shown)*

Ensembl ID	Gene	Description	AE Fold Change[a]	AE Adjusted *P* Value[b]	RE Fold Change[a]
ENSG00000262222	*AC133065.2**	RP11-876N24.4	4.09	0.00006	2.19
ENSG00000008853	*RHOBTB2*	Rho related BTB domain containing 2	2.96	0.0015	2.06
ENSG00000196482	*ESRRG*	estrogen related receptor-γ	2.48	0.0020	1.52
ENSG00000124784	*RIOK1*	RIO kinase 1	2.57	0.0030	1.83
ENSG00000146859	*TMEM140*	transmembrane protein 140	2.72	0.0036	1.84
ENSG00000092841	*MYL6*	Myosin light chain 6	0.46	0.0043	0.65
ENSG00000167333	*TRIM68*	Tripartite motif containing 68	0.32	0.0052	0.76
ENSG00000228794	*LINC01128*	long intergenic non-protein coding RNA 1128	2.05	0.0052	1.58
ENSG00000080189	*SLC35C2*	solute carrier family 35 member C2	0.36	0.0055	0.86
ENSG00000134324	*LPIN1*	Lipin	1.84	0.0057	1.14

All genes except for *AC133065.2* were observed 4 h postexercise. See Supplemental Table S2 for full list of uniquely differentially expressed genes. **AC133065.2* observed at both 1 and 4 h post exercise. [a]Fold change from baseline; [b]adjusted (Benjamini-Hochberg) *P* value.

highlight that different forms of exercise stimulate unique molecular activity in skeletal muscle.

Transcriptional Profile of Skeletal Muscle in Response to Acute Aerobic Exercise

Our transcriptome analysis identified 48 genes that were responsive only following acute AE during the postexercise time course (Supplemental Table S2). Interestingly, none of these genes were from mitochondrial DNA, which could be due to the early postexercise time course or to the moderate intensity of the exercise (vs. higher intensity) (22). Of particular interest, however, was the AE-specific increase in estrogen-related receptor-γ (ERRγ; *ESRRG*) observed 4 h postexercise. ERRγ is an orphan nuclear receptor commonly expressed in highly metabolic and vascularized tissues (28). Interestingly, *ESRRG* expression has also been shown to be higher in skeletal muscle comprised predominately of MHC I fibers compared with those comprised predominately of MHC II fibers (56). Consistent with our findings, *ESRRG* expression is increased in response to chronic low-frequency electrical stimulation in myotubes (27), and cross-sectionally, it has also been shown to be elevated in the muscle of trained vs. untrained individuals (27). Importantly, recent evidence utilizing a variety of transgenic mouse and cell culture models has identified that expression of *ESRRG* is associated with phenotypical changes highly aligned with AE training, including: improved mitochondrial function, greater oxidative capacity, improved endurance exercise, increased vascularization, a reduced respiratory exchange ratio, and a shift from MHC II to MHC I fibers (27, 41, 56, 63). Interestingly, the phenotypical changes associated with ERRγ could be independent of PGC-1α (56), which in the current study was upregulated during both the AE and RE trial. Although data from human skeletal muscle are limited, one cross-sectional study has shown that *ESRRG* expression in human skeletal muscle is positively correlated with MHC I fiber profile and $\dot{V}O_{2max}$ (27). In the current study, increased *ESRRG* expression at 4 h post AE was also accompanied by a gene cluster containing terms related to angiogenesis (Supplemental Table S3, AE cluster 6) as well as increased expression of *TMEM140*, which has been shown to be reduced under stimuli known to promote MHC II fiber type transitions (1). Collectively, ERRγ could represent an important molecule related to AE-specific adaptations in skeletal muscle.

In addition to specific targets discussed above, an interesting finding from our transcriptome analyses was the identification of an AE-specific gene cluster at 4 h containing terms related to ubiquitination (Supplemental Table S3, cluster 7). This finding is aligned with previous research indicating that AE appears to stimulate greater transcriptional activity of proteolytic genes relative to an acute bout of RE (42). In regard to the two well-described E3 ubiquitin ligases, we did not detect any change in atrogin-1 (*FBXO32*) expression during either exercise trial, whereas MuRF1 (*TRIM63*) expression was increased

Table 5. *Top 10 differentially expressed genes (by adjusted P value) responsive only to RE (not identified as statistically differentially expressed in response to AE at 1 h or 4 h; for comparative purposes, the fold change of these genes to AE is also shown)*

Ensembl ID	Gene	Description	RE Fold Change[a]	RE Adjusted *P* Value[b]	AE Fold Change[a]
ENSG00000133454	*MYO18B*	Myosin XVIIIB	1.83	3.52E-12	1.18
ENSG00000102313	*ITIH6*	Inter-α-trypsin inhibitor heavy chain family member 6	0.18	3.14E-09	0.49
ENSG00000128591	*FLNC*	Filamin C	2.68	4.10E-09	1.44
ENSG00000138678	*GPAT3*	Glycerol-3-phosphate acyltransferase 3	3.47	1.72E-08	1.63
ENSG00000006327	*TNFRSF12A*	TNF receptor superfamily member 12A	5.74	8.03E-08	2.39
ENSG00000137094	*DNAJB5*	DnaJ heat shock protein family (Hsp40) member B5	2.13	1.85E-07	1.31
ENSG00000140450	*ARRDC4*	Arrestin domain containing 4	4.87	5.17E-07	2.03
ENSG00000157119	*KLHL40*	Kelch-like family member 40	4.95	1.15E-06	2.41
ENSG00000140403	*DNAJA4*	DnaJ heat shock protein family (Hsp40) member A4	3.22	1.67E-06	1.64
ENSG00000152137	*HSPB8*	Heat shock protein family B (small) member 8	2.62	1.72E-06	1.55

All genes were observed at 4 h postexercise. See Supplemental Table S2 for full list of uniquely differentially expressed genes. [a]Fold change from baseline; [b]adjusted (Benjamini-Hochberg) *P* value.

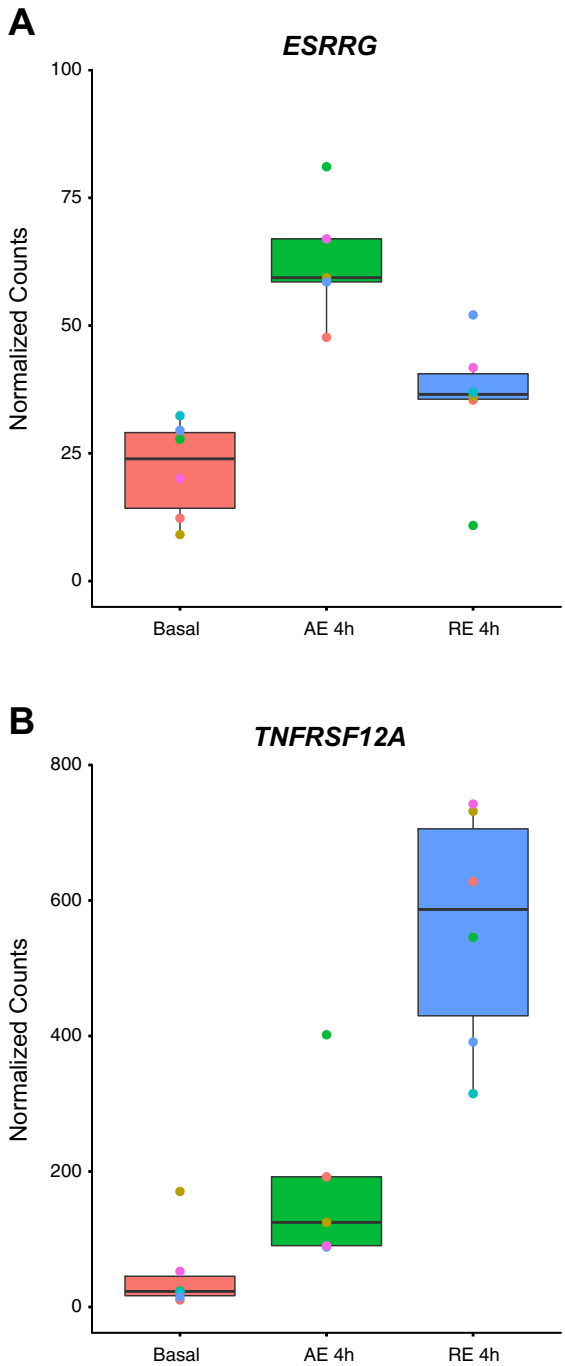

Fig. 3. Box plots displaying normalized gene counts for estrogen-related receptor-γ (*ESRRG*; *A*) and fibroblast growth factor receptor-14 (*TNFRSF12A*; *B*) in skeletal muscle at basal and at 4 h post-aerobic (AE) and -resistance exercise (RE). *ESRRG* was identified as the 3rd-most significant gene uniquely responsive to AE (Table 4), and *TNFRSF12A* was identified as the 5th-most significant gene uniquely responsive to RE (Table 5). Circles sharing the same color represent samples from the same participant.

in response to both the AE and RE trial, albeit to a slightly greater extent by AE (3.48- vs. 2.64-fold). However, two of the top 10 most significantly changed genes responsive only to AE were genes related to ubiquitination (*RHOBTB2* and *TRIM68*). In contrast, no genes with a role in ubiquitination were identified in the top 10 most significant genes responsive only to RE. Furthermore, although independent KEGG pathway analyses did not identify a uniquely enriched pathway in response to AE, an AE-specific gene cluster, including genes enriched in the FoxO pathway, a primary pathway stimulating protein ubiquitination (54), was identified at 1 h post-AE (Supplemental Table S3, cluster 4). Collectively, these data support previous findings (42) and further indicate that, relative to RE, acute AE appears to elicit a greater relative transcriptional response of genes related to proteolytic activity. Identification of the precise role and targets of AE-stimulated ubiquitination requires further study.

Transcriptional Profile of Skeletal Muscle in Response to Acute Resistance Exercise

An intriguing finding in the current study was the RE-specific induction of fibroblast growth factor receptor-14 (Fn14; *TNFRSF12A*). Using microarray, Raue et al. (65) originally identified *TNFRSF12A* to be very responsive to RE and highly correlated with muscle fiber growth. Subsequent research has also strongly supported *TNFRSF12A* to be highly responsive to RE (19, 49). In contrast, and consistent with our findings, Raue et al. (64) also demonstrated that skeletal muscle Fn14 (*TNFRSF12A* and protein) is much less responsive to acute running exercise over a 24-h postexercise time course, although different muscles were analyzed for each exercise mode. Fn14 is a tumor necrosis factor-like weak inducer of apoptosis (TWEAK) receptor that upon activation appears to stimulate NF-κB signaling (10). Indeed, although not identified as a significantly enriched pathway by KEGG analyses (adjusted P value = 0.18), RE altered expression of nine genes associated with NF-κB signaling. Interestingly, five of these genes were responsive only to RE (*TAB1*, *PTGS2*, *LBP*, *TNFRSF1A*, and *MAP3K14*), including MAP3K14 (*NIK*), which appears to be an upstream activator of the nonconical NF-κB pathway (46). Furthermore, *USP2*, which has also been identified as an important positive regulator of NF-κB signaling (50), was also specifically targeted by RE. Collectively, and coupled with previous findings (65), these data provide further support for Fn14 and NF-κB signaling as important targets of RE and potential mediators of RE-specific adaptations of skeletal muscle.

Generally, acute RE presented a much more robust stimulus for transcriptional activity within skeletal muscle as compared with AE. In addition to unique gene ontologies for biological processes related to endoplasmic reticulum stress and unfolded protein response (Supplemental Fig. S5), many of the most significant RE-specific genes (Table 5) have roles in regulating the structural integrity of the muscle fibers or are part of the heat shock protein response. It is well understood that the RE performed in this study consists of both concentric and eccentric muscle contractions, whereas cycling exercise consists primarily of concentric muscle contractions. Eccentric muscle action presents a more damaging stimulus to the structural integrity of muscle compared with concentric actions (62). To that end, heat shock proteins (HSPs) are known to respond to cellular stress, and they function to facilitate repair and restore cellular homeostasis (40). Furthermore, in addition to the HSPs listed in Tables 3 and 5, *HSPA1A*, the inducible member of the HSP70 family (a.k.a., HSP72), was increased only during the RE trial (4 h). Interestingly, overexpression of *HSPA1A* has

been shown to reduce muscle damage and accelerate muscle recovery following damaging exercise (47). The accelerated recovery with overexpression of *HSPA1A* may be related to role of HSP72 release in mediating the inflammatory response to damaging (eccentric) exercise, which is considerably delayed in *HSPA1A*-knockout mice (68). Collectively, the greater transcriptional response following acute RE could simply be related to a greater necessity to repair (or build) the cellular and structural environment as a result of the greater stress placed on the structural integrity of the muscle by performing unaccustomed eccentric contractions. On the other hand, the intriguing role for heat shock proteins (in particular HSP70/HSP72) for mediating skeletal muscle adaptations to exercise has recently been highlighted (32), and our transcriptome analyses identified that the transcriptional activity related to heat shock proteins is uniquely responsive to acute RE (in the untrained state). To what extent the heat shock protein response is related to managing the acute response to exercise or facilitating chronic adaptation, as previously discussed (32), requires further study.

Additional Study Considerations

We acknowledge the existence of some limitations. For instance, we recognize the preliminary nature of our findings given that our results are derived from a relatively small sample. In addition, given the crossover design, a single basal muscle biopsy was used to minimize the total number of biopsies required for each participant. We recognize that this approach may have impacted the findings, although the counterbalanced crossover design was used specifically to minimize this impact. We also focused on the acute rather than the chronic transcriptional response of skeletal muscle to AE and RE in the untrained state. Consequently, we cannot interpret our transcriptional profiles in relation to any exercise-specific phenotypical change occurring in skeletal muscle. However, in the setting of divergent exercise stimuli, our initial focus was on the acute transcriptional response to each exercise mode, given that it is suggested that the acute gene response to exercise is reflective of the specificity of skeletal muscle adaptation to exercise (26). In addition, the acute transcriptional response to exercise, as well as basal gene expression, can change in response to training (12, 65, 66, 71). Thus, future research is needed to more precisely identify the role of the acute vs. chronic transcriptional response to exercise on eliciting specific functional changes in skeletal muscle. In addition, the 4 h postexercise time point was chosen based on previously reported postexercise time course data (42, 80); however, we also recognize that that the time course for transcriptional activity in skeletal muscle after AE and RE can be extended longer (64). Therefore, we may not have identified all unique genes/pathways that may play a role in the exercise mode-specific response of skeletal muscle. Finally, our results were obtained from a single muscle and in response to a single form of AE. Additional research is necessary to determine whether similar responses would be observed in skeletal muscles with different functional characteristics and following different forms of aerobic exercise (i.e., running).

In summary, we present a preliminary examination of the immediate whole transcriptome response of human skeletal muscle to acute AE and RE. To our knowledge, this is the first investigation to utilize whole transcriptome RNA-seq to investigate the specific transcriptome response of human skeletal muscle to acute divergent exercise stimuli. Our data reveal several unique gene targets responsive to acute AE and RE. In particular, our results indicate and provide further support (27, 65) for consideration of the ERRγ-miRNA circuitry and TWEAK-Fn14-NF-κB signaling axis as potential key targets and mediators of the specific response of human skeletal muscle to AE and RE, respectively. Given the recent emergence of precision medicine and emphasis on the role of exercise (6, 58), these findings support the need for future research focused to better identify the role of exercise mode as it relates to targeting specific cellular skeletal muscle abnormalities. A better understanding of the unique processes stimulated by different forms of exercise has important implications for better preserving skeletal muscle health and for developing more effective exercise interventions to target a variety of skeletal muscle abnormalities that may be associated with skeletal muscle dysfunction.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.M.D., A.C.D., M.J.H., and C.C.C. conceived and designed research; J.M.D., A.C.D., M.A.N., A.L.S., A.J.W., D.P.C., and C.C.C. performed experiments; J.M.D., M.A.N., A.L.S., A.J.W., and M.J.H. analyzed data; J.M.D., A.C.D., M.A.N., and M.J.H. interpreted results of experiments; J.M.D. and M.A.N. prepared figures; J.M.D. and M.J.H. drafted manuscript; J.M.D., A.C.D., M.A.N., A.L.S., A.J.W., D.P.C., M.J.H., and C.C.C. edited and revised manuscript; J.M.D., A.C.D., M.A.N., A.L.S., A.J.W., D.P.C., and M.J.H. approved final version of manuscript.

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