J Appl Physiol 112: 1625–1636, 2012.
First published February 2, 2012; doi:10.1152/japplphysiol.00435.2011.

Transcriptome signature of resistance exercise adaptations: mixed muscle and fiber type specific profiles in young and old adults

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Submitted 8 April 2011; accepted in final form 31 January 2012

Raue U, Trappe TA, Estrem ST, Qian HR, Helvering LM, Smith RC, Trappe S. Transcriptome signature of resistance exercise adaptations: mixed muscle and fiber type specific profiles in young and old adults. *J Appl Physiol* 112: 1625–1636, 2012. First published February 2, 2012; doi:10.1152/japplphysiol.00435.2011.—This investigation examined the effects of acute resistance exercise (RE), progressive resistance training (PRT), and age on the human skeletal muscle Transcriptome. Two cohorts of young and old adults [*study A*: 24 yr, 84 yr (n = 28); *study B*: 25 yr, 78 yr (n = 36)] were studied. Vastus lateralis biopsies were obtained pre- and 4 h post-RE in conjunction with the 1st and 36th (last) training session as part of a 12-wk PRT program in *study A*, whereas biopsies were obtained in the basal untrained state in *study B*. Additionally, the muscle fiber type specific (MHC I and MHC IIa) Transcriptome response to RE was examined in a subset of young and old women from *study A*. Transcriptome profiling was performed using HG U133 Plus 2.0 Arrays. The main findings were *1*) there were 661 genes affected by RE during the 1st and 36th training bout that correlated with gains in muscle size and strength with PRT (termed the Transcriptome signature of resistance exercise adaptations); *2*) the RE gene response was most pronounced in fast-twitch (MHC IIa) muscle fibers and provided additional insight into the skeletal muscle biology affected by RE; *3*) skeletal muscle of young adults is more responsive to RE at the gene level compared with old adults and age also affected basal level skeletal muscle gene expression. These skeletal muscle Transcriptome findings provide further insight into the molecular basis of sarcopenia and the impact of resistance exercise at the mixed muscle and fiber type specific level.

microarray; Fn14; aging; skeletal muscle; fast-twitch

ADVANCED AGE IS ACCOMPANIED by a loss of skeletal muscle mass, strength, and function, a condition clinically known as sarcopenia (14). Although sarcopenia is characterized by a loss of muscle fibers and muscle fiber atrophy (24, 25), less is known about the etiology of sarcopenia. With the advancement of molecular biology tools, the gene expression profile of sarcopenia was first investigated a decade ago, where gene expression differences between young and old men were reported in the basal state (21). Since then, gene array studies of skeletal muscle of aged, healthy, and ambulatory individuals 66–76 yr of age have provided insight into the Transcriptome profile in older adults (17, 30, 39, 53, 54). However, given the accelerated loss of muscle mass and function observed in the 8th and 9th decade of life, microarray data from even older cohorts are needed (1, 11, 25).

Resistance exercise (RE) is an effective way to ameliorate age-related atrophy and increase skeletal muscle size and strength for individuals in their 60s and 70s (2, 22, 40, 49, 51, 55). We recently reported that free-living octogenarians (38, 43) appear to have blunted whole muscle, myocellular, and targeted gene expression adaptations to a resistance training program. Expanding on these cellular data to include genome-wide Transcriptome data would provide a more detailed view of the molecular events triggered by RE among young and old adults. To date, only one study has used microarray to evaluate the acute gene expression response to RE among young (37 yr) and old (73 yr) individuals (45). Twenty-four hours after a moderately damaging bout of RE, the Transcriptome response was greater among old compared with young and suggestive of an inflammatory response in the old that was not observed in the young participants. To provide further insight into the Transcriptome response after an RE stimulus known to induce hypertrophy if performed regularly, additional microarray data are needed in the first few hours after an RE stimulus when the Transcriptome appears to be the most affected (26, 59).

The overarching objective of the current investigation was to extend our previous research and provide additional insight into the Transcriptome of human aging skeletal muscle. Given our recent reports of an attenuated adaptation to resistance training among octogenarians at the whole muscle and myocellular level (38, 43), we were interested in the skeletal muscle Transcriptome response of these octogenarians to an acute bout of RE. We therefore conducted a microarray analysis on vastus lateralis tissue that was collected from young and old adults pre and 4 h post an acute bout of high-intensity RE at the beginning (1st training session) and at the end (36th training session) of a 12-wk-long resistance training program (Fig. 1). The study design allowed for examination of the acute Transcriptome response in the untrained and trained state and changes in the basal level Transcriptome pre- to posttraining among young and old adults. We also conducted unique correlation analyses, generating a set of genes that are responsive to acute RE (in the untrained and trained state) and correlate with increases in whole muscle size and strength after the 12-wk training program.

In addition, we conducted microarray analysis on isolated muscle fibers designated as either slow (myosin heavy chain, MHC I)- or fast-twitch (MHC IIa), which complemented the mixed muscle (multiple fiber types and inter-myocellular components) sample analysis. The fiber type specific data sets provide the first insights into the Transcriptome response of slow- and fast-twitch muscle fibers after a bout of RE. Fiber type specific genomic analyses are novel and particularly interesting given the differences in phenotype and plasticity between slow- and fast-twitch fibers in health, performance, and disease (7, 12, 22, 27, 29, 38, 48, 50). Last, we examined the effects of age on the basal level skeletal muscle Transcrip-

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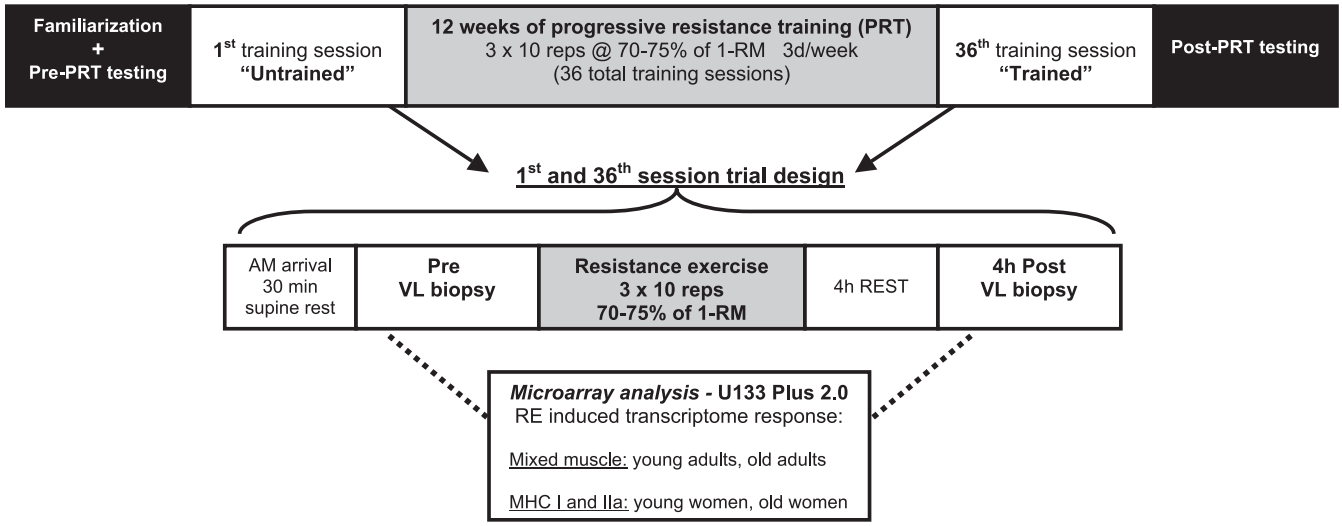

Fig. 1. Study design for the analysis of effects of resistance exercise (RE) and progressive resistance training (PRT) on vastus lateralis gene expression. Young and old adults of *study A* performed an acute bout of RE (3 × 10 repetitions at 70–75% of 1 repetition maximum) in the untrained and trained state. Muscle biopsies were collected pre and 4 h after the RE bout. In young and old women myosin heavy chain (MHC) I and IIa muscle fibers (pools of 20 fibers) were isolated and analyzed for gene expression response to RE.

tome by conducting microarray analysis on vastus lateralis tissue obtained from two separate aged (oldest to date) cohorts.

METHODS

Participants

A total of 64 individuals was included in this investigation: 16 young (8 men, 8 women) and 12 old (6 men, 6 women) individuals were included in *study A*, and 15 young (7 men, 8 women) and 21 old (10 men, 11 women) individuals were included in *study B* (Table 1). Participants in *study A* were recruited from the Muncie, Indiana, area, whereas participants from *study B* were recruited from the Little Rock, Arkansas, area. Elderly participants underwent a physical examination, which included medical history, blood, and urine samples for general health markers, resting and exercising electrocardiogram, and blood pressure. Participants were excluded if they had any acute or chronic illness, cardiac, pulmonary, liver, or kidney abnormalities, uncontrolled hypertension, insulin- or non-insulin-dependent diabetes, abnormal blood, or urine chemistries, arthritis, a history of neuromuscular problems, or if they smoked tobacco. We excluded individuals that had ever completed any formal exercise programs or physical activity outside of their activities of daily living. Following approval by the Institutional Review Boards (IRB), all procedures, risks, and benefits associated with the experimental testing were explained to the participants before they signed a consent form adhering to the guidelines of the IRB of the participating institutions.

The focus of *study A* was whole muscle and myocellular adaptations to progressive resistance training in young (20–30 yr) and very old (>80 yr) men and women (38, 43). A number of whole muscle function and myocellular tests were completed as part of *study B*, and these data and methods have been published elsewhere (19, 48). Both studies included measurements for muscle size, via computed tomography (CT) in *study A* and magnetic resonance imaging (MRI) in *study B*. Details for these measurements have been reported previously (38, 43, 48, 52). As can be seen in Table 1, the elderly participants had ~25% smaller muscle size and ~45% less whole muscle strength/power compared with their younger counterparts.

Experimental Design

The effects of acute RE and progressive resistance training (PRT) on the skeletal muscle Transcriptome was examined in *study A*. All participants of *study A* underwent a 12-wk PRT program (see *PRT in Study A*). Vastus lateralis (VL) muscle biopsies were collected in conjunction with the first and last training session, pre and 4 h post RE bout, which allowed for examination of the mixed muscle (i.e., non-fiber type specific homogenate) RE Transcriptome response in the untrained and trained state (Fig. 1). In a subset of young and old women of *study A* we assessed the feasibility of studying the effects

Table 1. *Subject characteristics from study A and study B*

	Study A			*Study B*		
	Young	Old	%Δ	Young	Old	%Δ
N (total = 64)	16	12		15	21	
Age, yr	24 ± 4	84 ± 3*		25 ± 3	78 ± 6*	
Weight, kg	72 ± 13	70 ± 9		72 ± 16	72 ± 12	
Height, cm	171 ± 11	165 ± 8		171 ± 10	168 ± 10	
BMI, kg/m^2	25 ± 5	26 ± 3		25 ± 4	25 ± 3	
Muscle strength[a] or power[b]	78 ± 26	47 ± 12*	−40	462 ± 157	247 ± 110*	−47
Muscle size, cm^2[c,d]	137 ± 25	104 ± 20*	−24	64 ± 14	49 ± 11*	−23

Data are mean ± SD. BMI, body mass index; [a]knee extension one-repetition maximum (kg) was measured in *study A*; [b]knee extension power (W) was measured in *study B*; [c]whole thigh muscle cross-sectional area (cm^2) was measured via computed tomography in *study A*; [d]quadriceps femoris muscle cross-sectional area (cm^2) was measured via magnetic resonance imaging in *study B*; %Δ percent difference between young and old subjects. *$P < 0.05$ from young within the same study.

of RE on the Transcriptome in isolated MHC I and IIa muscle fibers. The effects of age on the basal level skeletal muscle Transcriptome was examined by conducting mixed muscle gene expression profiling of VL muscle samples from young and old participants in *study A* and *study B* (Table 1). In both *study A* and *study B*, the subjects did not receive a standardized diet but were instructed to maintain their normal dietary habits for the duration of the study.

PRT in Study A

All participants in *study A* completed 12 wk of PRT (100% compliance), including 36 training sessions (3 days/wk) with three sets of 10 bilateral knee extensions at 70–75% of their one-repetition maximum (1 RM). This PRT protocol is very effective at inducing whole muscle strength (30–50%) and hypertrophy of the thigh muscles (5–10%) in individuals <80 yr of age (38, 49, 51, 56).

Muscle Biopsy

Muscle biopsies (6) of the VL were performed as previously described on each subject in *study A* (4 biopsies; pre and 4 h post RE during 1st and 36th training session) (36, 38) and *study B* (19, 48). In *study A*, the subjects arrived at the laboratory in the morning after an overnight fast and after a 30-min period of supine rest pre and 4 h post RE biopsies were obtained from contralateral legs. Similarly, the single biopsy in *study B* was obtained after a period of supine rest, but without any specific control on the timing of the subject's last meal. Each muscle sample was divided into longitudinal sections, and the tissue allocated for gene expression analysis was either placed in 0.5 ml of RNAlater (Ambion, Austin, TX) (*study A*) and stored at −20°C or frozen and stored in liquid nitrogen (*study B*) until RNA extraction.

Total RNA Extraction, Quality, and Quantity

Muscle samples from *study A* were transferred from RNAlater (Ambion) and placed in a mixture of 0.8 ml of RNA isolation reagent (TRI reagent) and 4 μl of PolyAcryl carrier (Molecular Research Center, Cincinnati, OH) and then homogenized. The frozen muscle samples from *study B* were immersed in RNAlater-ICE (Ambion) and stored at −20°C for 24 h before being homogenized in the aforementioned TRI-reagent mixture. As reported by our laboratory previously (26, 36, 37, 59, 60), total RNA was extracted from homogenized samples according to manufacturer's protocol. Each total RNA sample was purified using the RNeasy Micro Kit (Qiagen, Valencia, CA). The RNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and the quality was high for all samples as evidenced by the RIN numbers (mean ± SD) 7.6 ± 0.8 and 8.2 ± 0.3 for *study A* and *study B*, respectively. RNA concentration was determined using the Quant-iT RNA assay kit (Invitrogen, Carlsbad, CA) in conjunction with the Qubit fluorometer (Invitrogen).

Muscle Fiber Isolation, MHC Isoform Identification, and Total RNA Extraction of Individual Muscle Fibers

In young and old women of *study A*, a small muscle bundle from the pre and 4 h post RE muscle biopsies was placed in a Petri dish containing RNAlater (Ambion). Individual muscle fibers were separated under a light microscope, clipped, and handled, as reported by us previously (60). SDS-PAGE analysis, as detailed elsewhere (57), was used to determine the MHC isoform of each isolated single muscle fiber. Following MHC isoform identification, 20 pure MHC I fibers and 20 pure MHC IIa fibers, from each muscle sample, were selected for individual muscle fiber RNA extraction as described previously (60). The 20 RNA pellets of the same fiber type (either MHC I or MHC IIa) were combined and dissolved in 40 μl of DNase- and RNase-free water and stored overnight at 4°C before being transferred to −80°C.

Microarray Analysis

RNA labeling, subsequent microarray hybridization, fluidics, and scanning for all samples were performed by Expression Analysis (Durham, NC). Forty nanograms of total RNA from each mixed muscle sample (5 ng of total RNA for MHC I and IIa samples) was labeled using the NuGen WT-Ovation RNA Amp v2 kit and protocol (NuGen, San Carlos, CA). Each sample was hybridized to an Affymetrix Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA). Each microarray was washed and stained using an Affymetrix Fluidics Station 400 and scanned in an Affymetrix GeneChip Scanner 3000 7G scanner according to Affymetrix protocols. RNA samples from *study A* were analyzed independent from the samples of *study B*. Samples from both studies were processed in a randomized order to avoid potential systematic bias.

Microarray Analysis: MHC I and MHC IIa Sample Considerations

The current investigation is the first to conduct fiber type specific microarray analysis from human tissue. For this purpose we had MHC I and MHC IIa total RNA available from young and old women of *study A* (112 samples: 14 women × 4 biopsies × 2 fiber types). The MHC I and IIa samples contained total RNA from 20 muscle fibers, which is in accordance with our 20-fiber-pool model that has been used for targeted gene expression analysis (60) and protein synthesis measurements (12) previously. To initiate microarray sample processing, the samples were required to contain at least 1 ng/μl of total RNA. Ninety-eight samples (out of 112) had a total RNA concentration >1 ng/μl and were therefore shipped to Expression Analysis for sample processing (average [RNA] mean ± SD: 1.5 ± 0.4 ng/μl). RNA amplification (NuGen WT-Ovation RNA Amp v2 kit) was performed using 5 ng total RNA and 78 samples amplified successfully (resulted in >3.75 μg cDNA), whereas 20 samples did not amplify adequately (<3.75 μg cDNA). The average initial total RNA concentration of successfully amplified samples was (mean ± SD) 1.6 ± 0.4 ng/μl, whereas the initial concentration of failed samples was 1.3 ± 0.1. These data suggest the higher the initial starting RNA concentration, the more likely the amplification will be successful. There are several simple solutions to achieve a higher RNA concentration from fiber type specific samples, such as: *1*) include >20 muscle fibers, *2*) aim for a longer muscle fiber bundle, *3*) extract RNA from pooled muscle fibers as opposed to individual fiber RNA extraction (12), *4*) decrease the volume of RNAse free water added to RNA pellet at the end of RNA extraction.

Seventy-eight samples proceeded through the labeling protocol, after which 73 samples labeled properly and were therefore hybridized to U133 Plus 2.0 microarrays. In the end, the fiber type specific microarray analysis represent a subset of young (n = 4–6, depending on biopsy time point) and old (n = 2–5) women. The microarray quality control metrics for the fiber type specific samples suggested the microarray analysis was successful because ~48% of the microarray probesets were called present. For comparison, ~58% of the microarray probesets were called present in the mixed muscle samples. The difference in present calls between fiber type and mixed muscle samples can likely be explained by the mixed cell type profile (MHC I, MHC IIa, hybrid fibers, and intermyocellular components such as fibroblasts, neural, and vascular cells) of the mixed muscle samples. Collectively, the information provided here suggests that fiber type specific microarray analysis is very feasible as long as an initial total RNA concentration of >1.5 ng/μl is achieved.

Statistical Data Analysis

Collectively, 221 microarrays were analyzed; *study A* generated 112 mixed muscle microarrays (28 subjects × 4 biopsies = 112 samples) and 73 fiber type specific microarrays, whereas *study B* generated 36 mixed muscle microarrays. Hybridized microarrays were analyzed by Affymetrix microarray analysis software Microarray

Analysis Suite 5 (MAS 5). The microarray signals were normalized using the default method in Affymetrix MAS 5, but setting the target 2%-trimmed mean to 1,500 instead of 500. A series of data quality control assessments was conducted on all the microarray data as suggested by the manufacturer and Gautier et al. (16). Two mixed muscle samples (out of 148 samples) were excluded due to their low detection calls (less than median - 5%) and low correlation with other arrays (<0.9 vs. average 0.97). Three fiber type specific samples (out of 73 samples) were excluded from analysis due to their extreme low/high detection calls (one sample 29.6%, two samples with 70% vs. average 49%) and low correlation with other arrays (<0.7 vs. average 0.81). These excluded samples were clearly deviating from other samples in principal components analysis for each study.

For each probeset in *study B*, the normalized signal data were log-transformed and fitted to a linear regression model including age (young and old), sex (women and men), and their interaction. Sex was included in the model to account for gene expression differences between different sexes, although the study focused on the aging effect on muscle gene expression. A similar but more complicated model was utilized for *study A* by including time and its interaction with age and sex in the model (no sex effect in fiber type specific data, since all samples were from females) to incorporate pre and 4 h post RE during the 1st and 36th training session and the pre- and post-PRT (progressive resistance training program). A repeated-measurement analysis was carried out using a mixed model with the Kenward-Roger option in SAS (SAS Institute, Cary, NC). Correlation of observations from the same subject at different times were captured using first-order autoregressive covariance matrix. Fixed effect estimates were obtained from least squares means estimation of the fitted model and comparisons of interest were tested by *t*-test or *F*-test after specifying proper contrasts. The test results were transformed back to original scale to calculate fold change.

Next, to identify genes whose expression changed after an acute RE session and those that closely correlated with phenotypic change [i.e., muscle size (CSA) or muscle strength (1 RM) increase] after 12 wk of PRT, the gene expression data of each probeset (pre and 4 h post RE) during 1st or 36th training session were fitted to a linear model with subject and CSA or 1 RM strength. Each subject was treated as a fixed effect to allow different baseline gene expression in each subject. This test was completed to identify those genes whose fitted slope of CSA or 1 RM strength was similar in all subjects and significantly different from zero. Similarly, all the analysis was done in SAS using a mixed model procedure.

To reduce noise and false positives, only probesets with at least one call of "Present" or "Marginal" by MAS 5 and an average signal of at least one treatment group being 500 or larger were considered in the data analysis. The test *P* values from each comparison of all filtered probesets were adjusted for multiple testing using the false discovery rate (FDR) method (4). For the VL mixed muscle samples, probesets with FDR <0.1 and a fold-change >1.4 were considered as significant and followed up for further investigation. The fold-change cutoff of 1.4 was guided by our previous qPCR data (36, 37) (see below). Given the small number of subjects in the MHC I and IIa datasets, probesets with FDR <0.5 and a fold-change >1.4 were followed up for further investigation. The Ingenuity Pathway Analysis (IPA) system, version 8.5 (Ingenuity Systems, Redwood City, CA) was used for biological mapping analysis. The Affymetrix GeneChip/microarray data have been submitted to the GEO database (www.ncbi.nlm.nih.gov/geo/) for use by the scientific community: GSE28422, GSE28392, and GSE25941.

Microarray Data Verification

The microarray data captured among young and old men and women during the first training session (untrained RE response) in *study A* was compared with our previously published qPCR data in the same young and old women (36, 37). The same RNA was used for both the qPCR and microarray analysis. As can be seen in Table 2, the microarray data were in close agreement with the qPCR analysis.

Table 2. *Verification of microarray data*

	Microarray				qPCR	
Gene Symbol	Young Men	Young Women	Old Men	Old Women	Young Women	Old Women
Atrogin-1	NC	NC	1.8	1.8	NC	2.5
MuRF-1	1.4	1.6	1.3	1.4	3.6	2.6
Myogenin	1.4	1.5	NC	NC	1.7	NC
Myostatin	−2.9	−2.2	−2.3	−2.2	−2.0	−2.0
PDK4	2.1	4.0	3.0	2.5	9.7	4.4

Fold-changes observed in the microarray analysis among young and old men and women after resistance exercise in the untrained state were compared with the corresponding genes previously analyzed with qPCR in the same young and old women (36, 37). NC, no change.

RESULTS

Effects of RE on Skeletal Muscle Gene Expression

Transcriptome signature of resistance exercise adaptations. A correlation analysis was performed in which we examined the acute gene expression response to RE in the untrained and trained state and factored in the participant's physiological adaptation (i.e., muscle size and strength) to the 12-wk PRT program. All subjects (young and old) of *study A* were included in this analysis, with CSA changes ranging from −1.2 to +10.4 cm^2 and strength increases ranging from +5.7 to +41.3 kg (38, 43). There was a total of 661 annotated genes found to be altered with RE in the untrained and trained state that correlated with muscle size and strength changes with the 12-wk PRT program. More specifically, to qualify for this gene list, a gene had to pass our correlation filtering criteria (>1.4 fold, FDR <0.1) in both the untrained and trained state and correlate with muscle size and strength changes (Fig. 2). This list of 661 genes was termed the Transcriptome signature of resistance exercise adaptations. All 661 genes responded in the same direction when comparing the 1st and the 36th training sessions (e.g., if a gene was upregulated after the 1st training session, the same gene was also upregulated after the 36th training session). Figure 3 illustrates the acute gene response for two select genes (TNFRSF12A/Fn14 and NFKBIA/Iκβα) during the 1st and 36th (last) training session and how the response correlated with adaptations in 1 RM strength and CSA after 12-wk PRT. A more extensive example of genes included in the Transcriptome signature of resistance exercise adaptations is shown in Table 3, with a complete list in the online supplemental materials (S1). Canonical pathway analysis of this data set is also provided in online supplemental materials.

Young adults. When the subjects in *study A* were divided into young and old, the young participants showed a significant Transcriptome response to RE in both the untrained and trained state. There were a total of 1,165 annotated genes that changed expression (>1.4 fold, FDR <0.1) 4 h after RE in the untrained state (see online supplemental material S2). In the trained state, 644 annotated genes changed expression (>1.4 fold, FDR <0.1) 4 h after RE. Although the number of genes meeting the cutoff criteria in the untrained and trained states are different, the genes that made only one cutoff criteria have similar expression trend in both untrained and trained states. There were 524 genes that made the cutoff criteria in both the untrained and trained state (Table 4). The canonical pathway

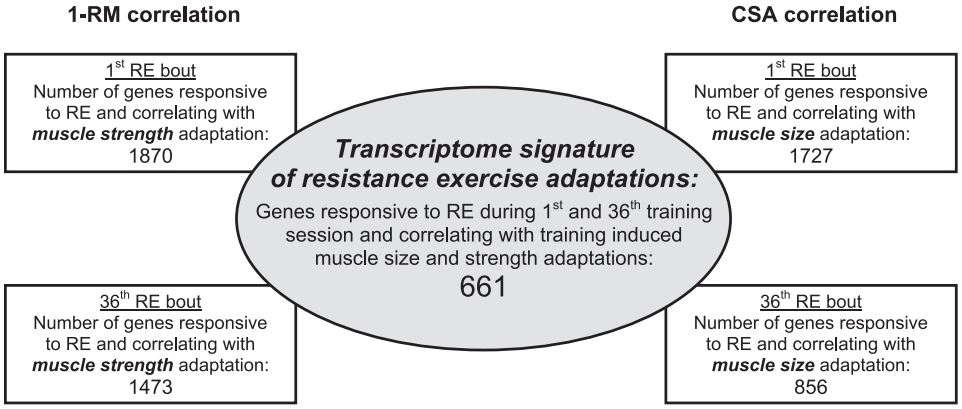

Fig. 2. Transcriptome signature of RE adaptations. A correlation analysis was conducted, examining each participant's (28 subjects, *study A*) gene response to resistance exercise in the untrained (1st training session) and trained (36th training session) state and the muscle size (CSA) and strength (1-RM) adaptations in each participant after 12 wk of training. If a gene was present in all 4 lists, it qualified for the Transcriptome signature of resistance exercise adaptations.

analysis of the untrained and trained gene response, as well as the overlap, is shown in online supplemental material S4.

Old adults. The old participants of *study A* also demonstrated a Transcriptome response to the RE, both in the untrained and trained state. There were a total of 595 annotated genes that changed expression (>1.4 fold, FDR <0.1) 4 h after RE in the untrained state and 569 genes changed expression after RE in the trained state (online supplemental material S2). There were 345 genes that made the cutoff criteria in both the untrained and trained state (Table 4). The canonical pathway analysis of the untrained and trained gene response, as well as the overlap, are shown in online supplemental material S4.

MHC I and MHC IIa muscle fiber Transcriptome response to RE. In a subset of young and old women of *study A* we were able to use the microarray technology to examine the effects of RE on the Transcriptome profile in MHC I and MHC IIa muscle fibers. The microarray analysis proved feasible for the genomic study of different fiber types using the 20 fiber pooled model (60). The MHC IIa fibers of the young women responded to RE with 463 annotated genes changing expression (>1.4 fold, FDR <0.5) 4 h after RE in the untrained state (Fig. 4). For comparison, in MHC IIa fibers among the old women, 63 genes changed expression (>1.4 fold, FDR <0.5) in response to RE in the untrained state. Very few genes were affected by RE among the MHC I fibers, with only 26 genes changing expression in young women and one gene responding in old women. In the trained state, the Transcriptome response was smaller among both young and old women in both fiber types. For a complete list of genes affected by RE in MHC I and IIa muscle fibers in the untrained and trained state see online supplemental material S3. The canonical pathway analysis of the young women's MHC IIa untrained response is shown in online supplemental material S4.

Among the 463 genes that responded to RE in the untrained state within the MHC IIa muscle fibers of the young women, 186 genes were in common (of which 96% were modulated in the same direction) with the genes found to respond to RE in young untrained mixed muscle (homogenized tissue). As can be seen in Table 5, the fold changes observed for RE-responsive genes within the MHC IIa dataset were most often (~97% of all genes) greater compared with corresponding genes within the mixed muscle dataset. It should be noted that the young mixed muscle data include both men and women, but the aforementioned comparison is justified because there were no apparent sex differences in the mixed muscle data. To illustrate the lack of sex differences and to provide a direct comparison for the MHC IIa data, Table 5 also includes a column showing the mixed muscle response of young women. There were 278 RE-responsive genes noted in the MHC IIa data set, which were not found to be modulated to a similar extent in mixed muscle (S3). The findings above highlight how fiber type specific Transcriptome analysis has the potential to provide new insight into the biology of skeletal muscle.

Considering the fiber type specific analysis was conducted in a subset of young and old women, we had insufficient samples to enable analysis of the MHC IIa muscle fiber RE-responsive genes in correlation with the physiological adaptation of these fibers with the 12-wk PRT program. We previously reported robust physiological adaptations within the MHC IIa fibers of the young women and blunted adaptations among the octogenarian women after PRT (38). As can be seen in Fig. 4, the quantitative Transcriptome response observed among the MHC IIa muscle fibers of the young and old women coincide with the MHC IIa hypertrophic response of these women and suggest a significant gene response is required to promote increases in muscle fiber size with resistance training.

Effects of 12-wk PRT on basal level skeletal muscle gene expression. Changes in basal level gene expression as a result of the 12-wk PRT program were examined in mixed muscle of both young and old adults of *study A*. Among the young adults, only 12 genes were found to be expressed differently after 12-wk PRT (>1.4 fold, FDR <0.1; Table 4 and online supplemental material S5) in the basal state. Among the old adults, 144 genes changed expression in the basal state as result of the 12-wk PRT program (>1.4 fold, FDR <0.1; Table 4 and online supplemental material S5) and seven of those genes were in common with the young adults. Thirty-four genes out of the 144 differentially expressed genes among the old were associated with cellular assembly and organization, such as organization of collagen and development of neurites (see online supplemental material S5).

Effects of Age on Basal Level Skeletal Muscle Gene Expression

Another objective of this investigation was to examine basal level gene expression among young and old adults. Age-related gene expression was examined in two separate large cohorts, which are the oldest reported to date, *study A* and *study B* (Table 1). There were a total of 88 annotated genes differentially expressed (>1.4 fold, FDR <0.1) between young and old individ-

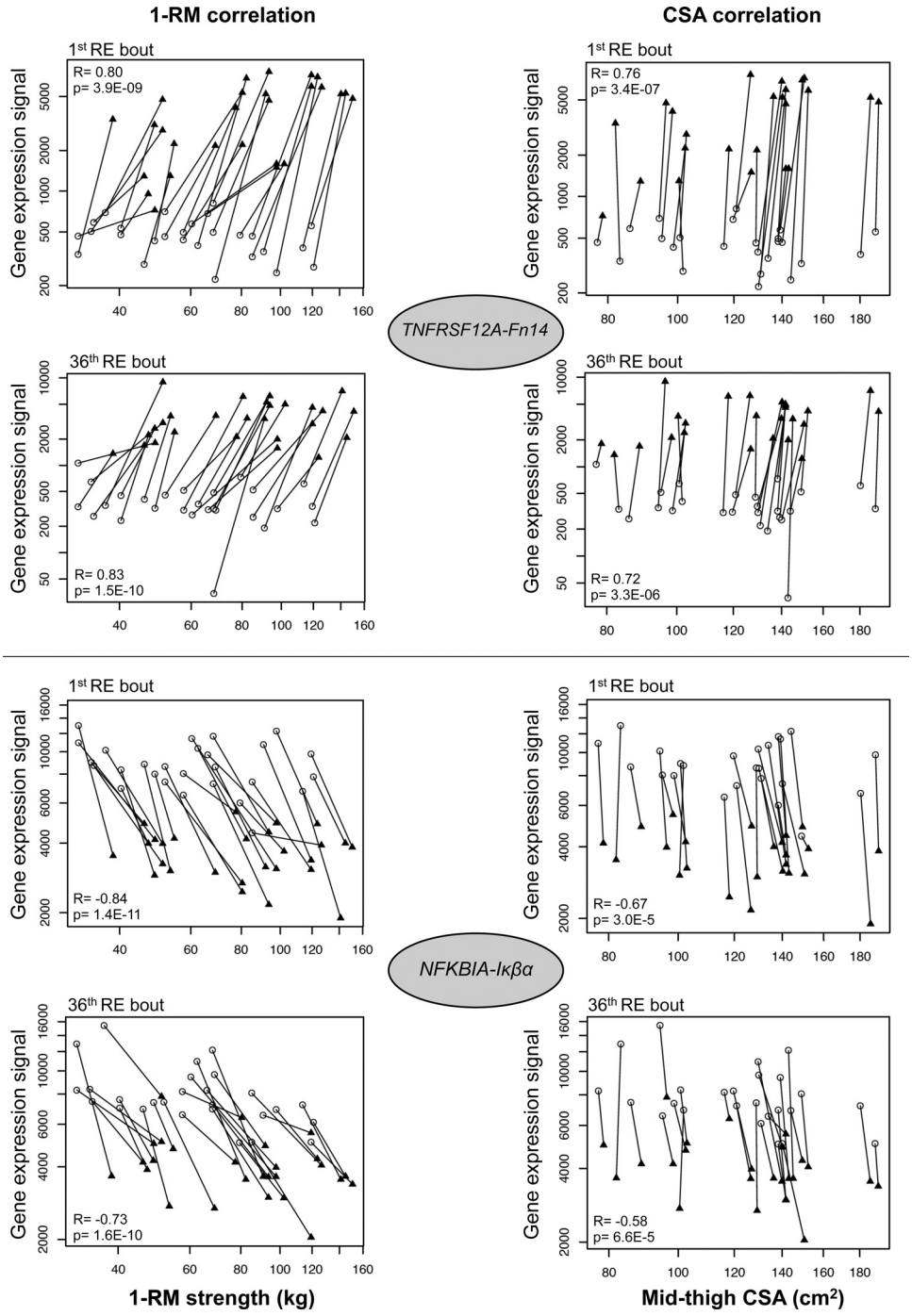

Fig. 3. Correlation illustration of 2 genes from the Transcriptome signature of resistance exercise adaptations. *Top* half: tumor necrosis factor receptor superfamily, member 12A (TNFRSF12A), also known as both fibroblast growth factor inducible gene 14 (Fn14) and the TWEAK receptor. *Bottom* half: NF-κB inhibitor alpha (NFKBIA), also known as Iκβα. Graphs show the responsiveness of these 2 genes within all the subjects during the 1st and 36th RE bout and how the gene response correlated with 1-RM and CSA adaptations after 12 wk of training. ○, pre (basal) value for the gene expression signal and 1-RM or CSA. ▲, 4 h post-RE gene expression signal and the 1-RM and CSA of the subject after 12 wk of training.

uals in *study A* (see online supplemental material S6). In *study B* a total of 416 annotated genes were found to be differentially expressed (>1.4 fold, FDR <0.1) between young and old adults (see online supplemental material S6). A comparison analysis of the two studies in IPA revealed 49 genes that were affected by age in both studies (Table 6). All 49 genes were regulated in the same direction (i.e., up or down) in both studies.

In addition, we investigated if any of these 49 genes were affected by age in a fiber type specific manner in the MHC I and IIa samples from the young and old women of *study A*. All 49 genes were expressed in both MHC I and MHC IIa muscle fibers, and a fiber type specific age effect was present in 19 of the 49 genes (Table 6). It should be noted that for a more direct comparison of the fiber type specific data to mixed muscle we also included mixed muscle data from the young and old women of these 49 genes. This additional analysis provided unique insight and suggests that some of the mixed muscle age-related differences can be attributed to the age difference primarily residing in MHC I and/or MHC IIa muscle fibers.

DISCUSSION

The purpose of this investigation was to study the effects of RE, PRT, and age on the skeletal muscle Transcriptome

Table 3. *A subset of genes in the Transcriptome signature of resistance exercise adaptations that are involved in various biological processes of skeletal muscle adaptations*

Symbol	Entrez Gene Name	1-RM correlation		CSA correlation	
		1st bout	36th bout	1st bout	36th bout
	Growth factor genes				
ANGPT1	angiopoietin 1	−0.58	−0.65	−0.60	−0.64
HBEGF	heparin-binding EGF-like growth factor	0.70	0.73	0.66	0.60
MSTN	myostatin	−0.62	−0.71	−0.55	−0.57
PDGFA	platelet-derived growth factor alpha polypeptide	0.41	0.53	0.40	0.46
VEGFA	vascular endothelial growth factor A	0.81	0.68	0.74	0.55
	Cell cycle and growth related genes				
CDK4	cyclin-dependent kinase 4	0.62	0.54	0.57	0.46
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	0.52	0.48	0.52	0.42
EIF4E	eukaryotic translation initiation factor 4E	0.56	0.58	0.58	0.44
GADD45G	growth arrest and DNA-damage-inducible, gamma	−0.78	−0.83	−0.60	−0.67
MYOG	myogenin (myogenic factor 4)	0.30	0.48	0.32	0.42
RICTOR	RPTOR independent companion of MTOR, complex 2	0.64	0.37	0.65	0.37
	Cytokine related signaling				
IL6R	interleukin 6 receptor	0.70	0.62	0.67	0.53
IL17RB	interleukin 17 receptor B	−0.46	−0.32	−0.42	−0.33
MAP3K14	mitogen-activated protein kinase kinase kinase 14 (aka NIK)	0.51	0.47	0.37	0.40
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α (aka Iκβα)	−0.84	−0.73	−0.67	−0.58
TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A (aka Fn14)	0.80	0.83	0.76	0.72
STAT3	signal transducer and activator of transcription 3	0.64	0.46	0.61	0.41
	Ubiquitin proteasome pathway related genes				
FOXO1	forkhead box O1	0.66	0.43	0.68	0.43
FOXO3	forkhead box O3	−0.85	−0.84	−0.71	−0.67
UBA7	ubiquitin-like modifier activating enzyme 7	−0.54	−0.37	−0.47	−0.28
UBR7	ubiquitin protein ligase E3 component n-recognin 7 (putative)	0.55	0.62	0.55	0.57
USP2	ubiquitin specific peptidase 2	0.80	0.74	0.74	0.62
USP25	ubiquitin specific peptidase 25	−0.41	−0.50	−0.48	−0.44
USP28	ubiquitin specific peptidase 28	0.64	0.64	0.60	0.54
USP40	ubiquitin specific peptidase 40	−0.47	−0.43	−0.46	−0.39
USP54	ubiquitin specific peptidase 54	0.46	0.54	0.43	0.49
	Substrate metabolism related genes				
HK2	hexokinase 2	0.45	0.43	0.42	0.38
PDK4	pyruvate dehydrogenase kinase, isozyme 4	0.59	0.39	0.55	0.28
PPARGC1A	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	0.85	0.72	0.70	0.58
PRKAG2	protein kinase, AMP-activated, gamma 2 noncatalytic subunit	0.84	0.85	0.73	0.63
	Amino acid transporters				
SLC15A4	solute carrier family 15 member 4	0.65	0.53	0.63	0.41
SLC36A1	solute carrier family 36 member 1	0.56	0.61	0.43	0.42
SLC38A1	solute carrier family 38 member 1	0.28	0.28	0.29	0.21
SLC38A4	solute carrier family 38 member 4	−0.29	−0.50	−0.29	−0.44
SLC3A2	solute carrier family 3 member 2	0.48	0.48	0.39	0.39
SLC7A8	solute carrier family 7 member 8	−0.57	−0.49	−0.50	−0.46

Correlation coefficient (R) values are given for the acute gene response during 1st and 36th training bout in correlation to 1-RM or CSA adaptations after 12-wk PRT. For a complete list of genes see online supplemental materials S1. All R values have an FDR <0.1.

profile in humans. The microarray data from this investigation profile the oldest cohorts to date in the literature, both with regards to basal and RE-induced gene expression. This investigation also provides the first Transcriptome signature of resistance exercise adaptations highlighting a set of genes that respond to RE and correlate with physiological adaptations of muscle size and strength after a training program. Additionally, we provide the first microarray data on muscle fiber type (slow vs. fast twitch) specific gene responses to RE and show that this approach can provide further insights to the study of the skeletal muscle Transcriptome.

Transcriptome Signature of Resistance Exercise Adaptations

One of the main objectives of this investigation was to examine the gene expression response to a regular nondamaging bout of RE (60), because it has been suggested the gene response after acute exercise reflects the specificity of the muscular adaptation to the exercise (15). In the current investigation we had a unique opportunity to relate the acute gene response after RE in 28 individuals to their whole muscle outcomes after a training period. The acute gene response to RE in the untrained and trained state was captured at 4 h

Table 4. *Number of genes responding to an acute bout of resistance exercise (RE) in the untrained and trained state in young (24y) and old (84y) adults of Study A*

	Untrained response to acute RE	Common genes UT and T	Trained response to acute RE	Basal response to PRT
Young adults	1165	524	644	12
Common genes young and old	449		349	7
Old adults	595	345	569	144

RE, resistance exercise. Number of genes in common between the untrained (UT) and trained (T) response or common between young (Y) and old (O) adults are provided. Basal response indicates the number of genes that changed expression in the basal state after 12 wk of progressive resistance training (PRT).

post-RE, a time point carefully chosen based on our previous time course investigations with the current RE protocol (26, 59). The data generated created the first Transcriptome signature of resistance exercise adaptations (TSREA) and includes 661 genes (see online supplemental material S1). In line with the anabolic nature of the RE stimulus, the cellular pathways affected by the RE appeared to be largely growth related (see online supplemental material S4).

A number of notable genes in the TSREA data set are shown in Table 3, among which the fibroblast growth factor inducible gene 14 (Fn14), also known as TNFRSF12A or the TWEAK receptor, was of particular interest. Fn14 was induced after RE in mixed muscle of all subjects (Fig. 3) and in MHC IIa muscle fibers, which is in line with two recent reports (13, 18) suggesting Fn14 is required for the expression of myogenic regulatory factors and differentiation of myoblasts into myotubes. Fn14 mRNA has been shown to increase in response to skeletal muscle tissue injury (33) and denervation-induced atrophy (32) in rodents, mechanical stretch of cardiac tissue (10, 34), and by growth factors such as PDGF and VEGF (both growth factors were induced in TSREA) (9). The cytokine TWEAK is the ligand for Fn14, although it has been suggested that changes in Fn14 expression alone has a greater impact during skeletal muscle regeneration (13, 33) and denervation-induced atrophy (32). In support of Fn14 expression being the main regulator of TWEAK/Fn14 pathway activity in skeletal muscle, TWEAK gene expression was not affected by RE in the current study. We detected TWEAK in all 148 mixed muscle and 73 MHC I and IIa samples, although major sources of this cytokine are reported to be the leukocytes including macrophages and neutrophils (8).

The TWEAK/Fn14 pathway is evolutionarily ancient and activates the multifaceted NF-κB pathway (9, 32). We have several data points in the TSREA and MHC IIa datasets that suggest the NF-κB pathway was activated by our RE protocol. First, we observed an induction of the NF-κB inducible kinase (NIK), also known as MAP3K14. NIK phosphorylates IKKβ, which in turn phosphorylates the NF-κB inhibitor Iκβα, which subsequently releases NF-κB and allows for NF-κB nuclear entry, whereas Iκβα is targeted for degradation (42). We observed a decrease in Iκβα gene expression in all subjects (Fig. 3) after RE, and this decrease likely promotes NF-κB pathway activity. In addition, there was an induction of ubiquitin specific peptidase 2 (USP2), which recently was suggested to be crucial for full NF-κB pathway activation (31). The transcription factor NF-κB has a vast number of target genes (35), many of which were modulated in our TSREA dataset. Interestingly, the Fn14 gene was recently shown to be among the NF-κB target genes (58). In support of our findings, exercise is a known activator of the multifaceted NF-κB pathway in both humans and animal models, although the mechanisms of activation and ultimate function with regards to exercise are not completely understood (23). Collectively, our data suggest a possible mechanism of NF-κB pathway activation is through the induction of Fn14 as a result of minor tissue disruption and mechanical stretch of skeletal muscle tissue after high-intensity RE. Although the NF-κB pathway is most often associated with inflammation, immunity, stress response, and proteolysis, our Transcriptome data indicate that NF-κB pathway activation is part of the normal and coordinated physiological response to RE.

Transcriptome Response to Resistance Exercise Among Young and Old Adults

We also separated the participants of *study A* into young and old cohorts to better understand how age impacts the RE-induced Transcriptome response. Acute RE had a significant impact on VL gene expression among the young adults in both

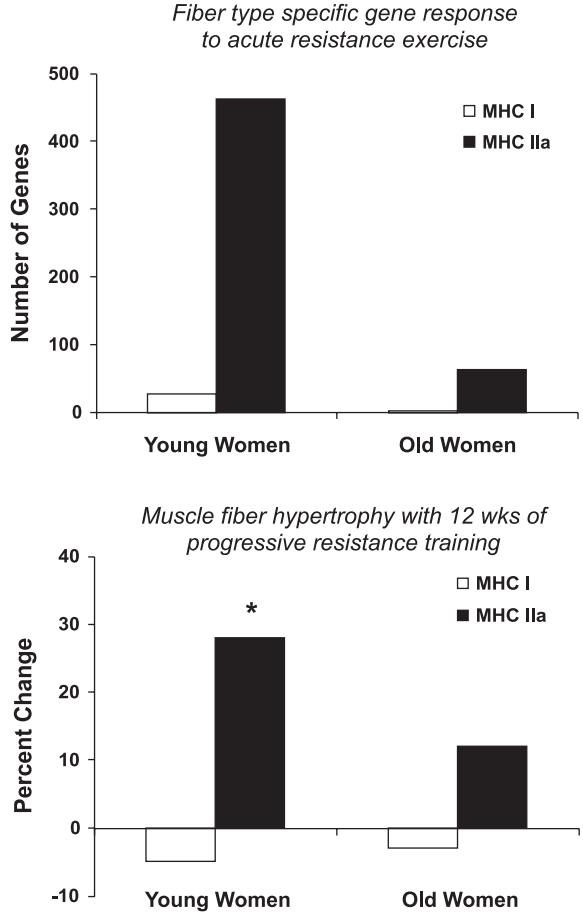

Fig. 4. Fiber type specific microarray analysis revealed fast-twitch MHC IIa muscle fibers of young women are responsive to acute RE in the untrained state with 463 genes altering expression, whereas only 63 genes responded among the MHC IIa muscle fibers of the old women (*top*). When the fiber type specific gene response of the young and old women is coupled with the muscle fiber CSA adaptations after PRT (*bottom*) in those women (38), the data support that a substantial gene response after RE is needed to acquire greater physiological adaptations over time. *$P < 0.05$ from pre- to posttraining.

Table 5. *MHC IIa and mixed muscle gene response to acute resistance exercise (1st RE bout)*

Symbol	Entrez Gene Name	*MHC IIa YW* (4 h post/pre)	*Mixed Muscle YW* (4 h post/pre)	*Mixed Muscle Young* (4 h post/pre)
HBEGF	heparin-binding EGF-like growth factor	7.0	4.7	4.5
VEGFA	vascular endothelial growth factor A	2.6	1.8	1.8
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	4.9	7.1	4.8
GADD45G	growth arrest and DNA-damage-inducible, gamma	−14.7	−4.2	−3.8
RICTOR	RPTOR independent companion of MTOR, complex 2	4.1	1.8	1.7
IL6R	interleukin 6 receptor	4.1	3.0	2.8
MAP3K14	mitogen-activated protein kinase kinase kinase 14 (NIK)	3.3	1.9	1.7
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (Iκβα)	−2.8	−2.7	−2.5
TNFRSF12A	tumor necrosis factor receptor superfamily, member 12A (Fn14)	22.5	11.6	11.9
FOXO1	forkhead box O1	3.7	2.0	1.8
FOXO3	forkhead box O3	−6.1	−2.9	−2.7
USP2	ubiquitin specific peptidase 2	3.6	1.7	1.7
USP28	ubiquitin specific peptidase 28	2.6	1.6	1.7
HK2	hexokinase 2	4.6	2.1	2.2
PDK4	pyruvate dehydrogenase kinase, isozyme 4	32.5	4.0	2.9
PPARGC1A	peroxisome proliferator-activated receptor gamma, coactivator 1 α	3.7	2.5	2.2
PRKAG2	protein kinase, AMP-activated, gamma 2 noncatalytic subunit	10.1	6.2	6.6

Data are shown as a fold ratio 4 h post-RE/pre. Gene response was generally more pronounced in MHC IIa muscle fibers from the young women (YW) compared with mixed muscle of the YW and Young (mixed sex). Table includes a sample of responsive genes related to growth, cytokine signaling, protein breakdown, and substrate metabolism. Genes listed above are also present in the Transcriptome signature of resistance exercise adaptations (see Table 3). A complete list of RE responsive genes for MHC IIa can be seen in online supplemental material S3 and for mixed muscle in online supplemental material S2.

the untrained and trained state (Table 4). Considering our statistical filters, this is the most robust (in terms of number of genes) RE response reported in the literature to date, notably as a result of only 30 high-intensity contractions. Previous acute RE microarray investigations have employed more vigorous protocols such as 300 maximal eccentric contractions (28) or 80 concentric-eccentric contractions (45, 61), which resulted in muscular damage (28, 45). Overall comparisons to other studies are difficult because there are many factors influencing microarray data, such as exercise bout, timing of postexercise biopsy, number of subjects, feeding status, RNA quality, microarray platform, and statistics employed.

We were particularly interested in the RE microarray data from the octogenarians, because these individuals had shown blunted whole muscle and cellular adaptations (38, 43) to an otherwise effective PRT program (49, 51, 56, 57). The octogenarian participants also demonstrated a large gene expression response 4 h after RE in the untrained and trained state, although fewer genes were modulated compared with young adults (Table 4). It is noteworthy that for a given gene, the young adults often had a greater fold change compared with old adults (see online supplemental material S2). We do not believe that MHC distribution of the participants played a role in the differences observed between young and old adults because there was no difference in MHC distribution among the groups (see online supplemental material S2). It is plausible that the time course for changes in gene expression after RE is delayed in the elderly, which is supported by Thalacker-Mercer et al. (45) who reported a greater Transcriptome response in old compared with young at 24 h post-RE, although their RE protocol (80 repetitions) was different from ours (30 repetitions). The biological interpretation of RE-responsive genes among young and old revealed many similarities along with some differences (see online supplemental material S4). Nevertheless, when considering the blunted skeletal muscle adaptations among the octogenarian after the PRT program (38, 43), our data suggest the quantity and magnitude of the acute gene expression response after an RE bout may influence the overall adaptive response to a training program. This finding supports previous reports of gene changes being greater in high responders compared with low responders with an endurance training program (46, 47) or resistance training program (3).

MHC I and MHC IIa muscle fiber Transcriptome response to resistance exercise. For the first time, we present microarray analysis on RNA material extracted from pooled (20 muscle fibers) MHC I or IIa muscle fibers. The data presented here show a significant Transcriptome response within the fast-twitch MHC IIa muscle fibers (463 genes) of the young women after RE in the untrained state, whereas a limited response was observed in the MHC IIa fibers (63 genes) of the old women. These data complement our previous report of MHC IIa hypertrophy (CSA +28%) among these young women and a lack of MHC IIa hypertrophy in the old women after 12 wk of training (38) (Fig. 4). There were many similarities in RE affected biology (biological interpretation indicated many growth-related processes) between mixed muscle of the young and MHC IIa muscle fibers in the untrained state, further supporting the quality of the MHC IIa dataset and that MHC IIa muscle fibers are targeted during RE. It is also noteworthy that the aforementioned Fn14-NF-κB cascade appears activated in MHC IIa muscle fibers. Gene fold-changes observed within the MHC IIa fibers were often (97% of common genes) greater than the fold-changes of corresponding genes in the mixed muscle data set (Table 5). This difference suggests a greater fidelity may be achieved with fiber type specific microarray analysis as opposed to mixed muscle in which fiber type specific changes may be masked by the inclusion of several fiber types and intermyocellular (e.g., fibroblasts, leukocytes, vascular, and neural cells) components. The fiber type specific microarray approach provides new opportunities to investigate muscle fiber type specific health at the Transcriptome level. This is an important and emerging area because each muscle fiber type contributes differently to muscle per-

Table 6. *49 genes differentially expressed in the basal state between young and old adults in both study A and B (FDR <0.1)*

Symbol	Entrez Gene Name	*Study A* (old/young)	*Study B* (old/young)	Women of *Study A*		
				Mixed Muscle (OW/YW)	MHC I (OW/YW)	MHC IIa (OW/YW)
ATP1B4	ATPase, (Na^+)/K+ transporting, beta 4 polypeptide	1.6	1.6	—	2.0	4.2
BEX2	brain expressed X-linked 2	3.1	2.3	3.8	3.4	—
C1QB	complement component 1, q subcomponent, B chain	2.4	2.3	3.3	3.0	—
CCNG2	cyclin G2	1.6	1.7	1.9	—	—
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	5.6	3.4	9.5	2.8	3.5
CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	2.3	2.2	2.3	—	—
CLIC5	chloride intracellular channel 5	−1.5	−1.5	−1.3	—	—-
COL19A1	collagen, type XIX, alpha 1	7.9	2.7	10.3	—	—
COL4A6	collagen, type IV, alpha 6	1.8	1.8	1.5	—	—
COL5A3	collagen, type V, alpha 3	−1.7	−1.5	−1.7	—	—
DAAM2	dishevelled associated activator of morphogenesis 2	−1.5	−1.8	—	—	—
DDB2	damage-specific DNA binding protein 2, 48 kDa	1.7	1.9	2.4	—	—
DENND4A	DENN/MADD domain containing 4A	1.5	1.4	1.7	—	—
DMRT2	doublesex and mab-3 related transcription factor 2	−4.9	−3.1	−3.8	−4.1	−6.6
EPB41L3	erythrocyte membrane protein band 4.1-like 3	2.2	1.8	2.2	—	—
EPB41L4B	erythrocyte membrane protein band 4.1 like 4B	2.6	2.4	3.8	—	—
FAM171A1	coagulation factor XIII, A1 polypeptide	−1.8	−1.8	−1.8	—	—
F13A1	family with sequence similarity 171, member A1	2.5	2.2	2.7	3.1	—
FEZ2	fasciculation and elongation protein zeta 2 (zygin II)	1.9	2.0	1.3	2.5	—
FLRT2	fibronectin leucine rich transmembrane protein 2	1.9	1.7	1.8	3.8	3.8
FOLR2	folate receptor 2 (fetal)	2.2	1.5	2.5	—	—
FST	follistatin	2.3	2.0	2.2	—	—
GREB1	growth regulation by estrogen in breast cancer 1	−1.8	−1.5	−3.2	—	−49.7
HOXB2	homeobox B2	−2.1	−2.0	−2.1	—	−2.6
ITGA9	integrin, alpha 9	2.2	1.5	2.5	—	—
KCNJ16	potassium inwardly-rectifying channel, subfamily J, 16	6.0	3.0	10.8	—	—
KLF5	Kruppel-like factor 5 (intestinal)	1.8	1.5	2.0	2.6	3.0
LGI1	leucine-rich, glioma inactivated 1	1.9	2.7	—	—	—
LTBP2	latent transforming growth factor beta binding protein 2	1.5	1.4	1.4	—	—
LYVE1	lymphatic vessel endothelial hyaluronan receptor 1	2.7	2.3	2.9	3.1	—
MS4A4A	membrane-spanning 4-domains, subfamily A, member 4	2.2	2.4	2.5	—	—
MYH8	myosin, heavy chain 8, skeletal muscle, perinatal	6.9	2.9	5.5	—	—
NMNAT1	nicotinamide nucleotide adenylyltransferase 1	−1.5	−1.6	−1.4	—	—
NT5C2	5′-nucleotidase, cytosolic II	1.5	1.6	1.5	4.0	—
PCDH9	protocadherin 9	2.0	1.8	2.7	—	—
PLAG1	pleiomorphic adenoma gene 1	3.4	2.3	3.9	—	5.9
RAB11FIP3	RAB11 family interacting protein 3 (class II)	1.6	1.4	1.5	—	16.7
RP13-102H20.1	hypothetical protein FLJ30058	9.7	5.0	11.4	16.9	17.3
RP5-1022P6.2	hypothetical protein KIAA1434	−1.6	−1.6	−1.5	—	—
RXRG	retinoid X receptor, gamma	−1.5	−1.4	—	−1.8	−3.6
SKAP2	src kinase associated phosphoprotein 2	2.1	1.7	2.2	—	—
SLC16A6	solute carrier family 16, member 6	2.0	2.2	2.3	—	—
SLC38A1	solute carrier family 38, member 1	−2.7	−1.8	−2.0	−4.3	—
SLIT2	slit homolog 2 (Drosophila)	1.6	1.4	1.8	—	—
SLPI	secretory leukocyte peptidase inhibitor	3.1	2.3	3.4	—	—
SYNPO2	synaptopodin 2	1.9	2.1	1.9	3.5	—
TMEM176B	transmembrane protein 176B	1.7	1.6	2.1	—	—
TPPP3	tubulin polymerization-promoting protein family member 3	2.0	1.4	1.7	—	—
ZNF385B	zinc finger protein 385B	1.7	1.8	—	—	—

Data are shown as a fold ratio old/young. For a complete list of genes affected by age within *study A* or *study B* see online supplemental material S6. The 49 genes were also examined in the MHC I and MHC IIa data sets and the mixed muscle data set generated from young (YW) and old women (OW) of *study A* to see if any of the 49 genes were significantly ($P < 0.05$) affected by age in either fiber type. (-) gene present, but the fold ratio was not significant ($P > 0.05$). The FDR approach was not applied to the women's data presented here since the comparison was limited to 49 genes as opposed to the whole genome.

formance, which has profound implications for human locomotion, athletic performance, and numerous clinical conditions such as sarcopenia, cachexia, and muscle-associated metabolic disorders.

MHC I muscle fibers demonstrated a very limited gene response in both young and old women after RE, which supports our previous finding that MHC I muscle fibers show limited plasticity in size and contractile function in response to 12 wk of PRT in these women (38). Collectively, the fiber type specific microarray data complement the mixed muscle data and also support that a substantial Transcriptome response after each training bout may be necessary for a physiological adaptation to take place over time (15).

Effects of Age on the Basal Level Transcriptome

The final aim of this investigation was to gain insight into the basal Transcriptome profile associated with sarcopenia.

The comparison of two independent datasets provided an extra level of verification rarely seen with gene expression profiling studies. There was a greater number of genes affected by age in *study B* compared with *study A*, and at this point it is not clear what caused this difference. Potential factors include the regions (AR vs. IN) from which the subjects were recruited and a subtle age difference of the subjects. In *study B*, the majority of the elderly (15 out of 21) subjects were in their 70s, and it is possible that these individuals were in a different atrophy phase compared with the octogenarians in *study A*. We did find 49 genes that were affected by age and regulated similarly in both *study A* and *study B* (Table 6). In addition, we show how these 49 genes are affected by age in MHC I and MHC IIa muscle fibers. Some of the 49 genes have also been identified as affected by age in previous reports (17, 30, 53, 54), further validating these genes as sarcopenia-associated genes. Perhaps most notable among the 49 age-related genes were three different cell-cycle inhibitors (CDKN1A, CDKN2B, and CCNG2) (5, 41) that were all upregulated in the elderly, suggesting that the cell-cycle events of aged skeletal muscle may be inhibited and that cell cycle arrest and subsequent differentiation as part of an attempt at regeneration may be underway to attenuate the ongoing atrophy. CDKN1A (also called P21), in particular, was upregulated 3.4- and 5.6-fold, and this is in agreement with previous studies (30, 53, 54). We show for the first time that the age-related upregulation of CDKN1A is present in both MHC I and MHC IIa muscle fibers. Interestingly, CDKN1A was also upregulated with RE among the old participants, which is in line with its suggested role in skeletal muscle regeneration (20).

Summary

The data presented here provide one of the most comprehensive microarray datasets on human skeletal muscle to date, with a focus on gene regulation with resistance exercise, progressive resistance training, age, and muscle fiber type. Our data suggest that an RE bout consisting of only 30 high-intensity contractions has a substantial impact on the skeletal muscle Transcriptome, and these gene responses were put into unique context when correlated with physiological adaptations from the 12-wk PRT program. A Transcriptome signature of resistance exercise adaptations was generated that included 661 genes responsive to RE and correlative with increases in whole muscle size and strength after PRT. The fiber type specific microarray analysis suggested the RE-induced gene response was targeted to the fast-twitch muscle fibers, and new insight into the biology of skeletal muscle was gained from this novel analysis. The biological interpretation of the RE-responsive genes showed evidence of an anabolic stimulus, which support the physiological adaptations typically observed with RE (2, 22, 38, 40, 44, 51).

Future skeletal muscle research should consider these Transcriptome data, which highlight how potent an exercise stimulus can be to the molecular milieu of skeletal muscle. More in-depth investigation is warranted for several aspects of the data, including the TWEAK/Fn14 pathway, to learn more about its role in human skeletal muscle mass regulation in health and disease. Collectively, the skeletal muscle Transcriptome findings presented here provide further insight into the molecular basis of sarcopenia and the impact of resistance exercise at the mixed muscle and fiber type specific level.

GRANTS

Research support provided by National Institutes of Health grants (AG15833, AG00831, and AG18409) and Eli Lilly and Company.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: U.R., T.A.T., S.T.E., H.-R.Q., L.M.H., R.C.S., and S.W.T. conception and design of research; U.R., T.A.T., and S.W.T. performed experiments; U.R., S.T.E., and H.-R.Q. analyzed data; U.R., T.A.T., S.T.E., H.-R.Q., L.M.H., R.C.S., and S.W.T. interpreted results of experiments; U.R. and H.-R.Q. prepared figures; U.R., T.A.T., and S.W.T. drafted manuscript; U.R., T.A.T., S.T.E., H.-R.Q., L.M.H., R.C.S., and S.W.T. edited and revised manuscript; U.R., T.A.T., S.T.E., H.-R.Q., L.M.H., R.C.S., and S.W.T. approved final version of manuscript.

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