Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy


1Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden; 2Department of Biosciences and Nutrition, Karolinska Institute, Huddinge, Sweden; 3Department of Neurology, University of Iowa, Iowa City, Iowa; 4Byrd Health Science Center, West Virginia University, Morgantown, West Virginia; and 5School of Education, Health, Human Performance, and Recreation, Baylor University, Waco, Texas

Submitted 18 December 2013; accepted in final form 21 January 2014

Nader GA, von Walden F, Liu C, Lindvall J, Gutmann L, Pistilli EE, Gordon PM. Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy. J Appl Physiol 116: 693–702, 2014. First published January 23, 2014 doi:10.1152/japplphysiol.01366.2013.—We sought to determine whether acute resistance exercise (RE)-induced gene expression is modified by RE training. We studied the expression patterns of a select group of genes following an acute bout of RE in naïve and hypertrophying muscle. Thirteen untrained subjects underwent supervised RE training for 12 wk of the nondominant arm and performed an acute bout of RE 1 wk after the last bout of the training program (training + acute). The dominant arm was either unexercised (control) or subjected to the same acute exercise bout as the trained arm (acute RE). Following training, men (14.8 ± 2.8%; P < 0.05) and women (12.6 ± 2.4%; P < 0.05) underwent muscle hypertrophy with increases in dynamic strength in the trained arm (48.2 ± 5.4% and 72.1 ± 9.1%, respectively; P < 0.01). RE training resulted in attenuated anabolic signaling as reflected by a reduction in rpS6 phosphorylation following acute RE. Changes in mRNA levels of genes involved in hypertrophic growth, protein degradation, angiogenesis, and metabolism commonly expressed in both men and women was determined 4 h following acute RE. We show that RE training can modify acute RE-induced gene expression in a divergent and gene-specific manner even in genes belonging to the same ontology. Changes in gene expression following acute RE are multidimensional, and may not necessarily reflect the actual adaptive response taking place during the training process. Thus RE training can selectively modify the acute response to RE, thereby challenging the use of gene expression as a marker of exercise-induced adaptations.

gene expression; hypertrophy; resistance exercise; skeletal muscle; training

RESISTANCE EXERCISE-INDUCED skeletal muscle hypertrophy develops following repeated exercise bouts (i.e., the training effect) (13), and this adaptive response results from the cumulative responses to each individual exercise bout (i.e., the acute response) (8). Notably, although this notion, based on the principle of repetition is widely proven and empirically accepted, little is known about the molecular mechanism(s) underpinning RE training-induced adaptations. One hypothesis is that following each exercise bout, an acute transcriptional response occurs that will be of higher magnitude following the successive bouts (5). In turn, this will result in the accumulation of cognate proteins (gain of function), thereby enabling or augmenting key biological processes needed for adaptation (9, 16, 40). Although this is theoretically sound, the principle of repetition during RE training remains to be explained at various levels (i.e., DNA modifications, mRNA levels, protein levels, posttranslational modifications).

Recent high-throughput studies have begun to elucidate the transcriptional responses to RE training (31, 39, 44). However, the diverse study designs or sample collection timing precludes the determination of how the expression of specific target genes is modified during the training process and/or ensuing phenotypes. Knowledge gained from endurance exercise (EE) studies demonstrates that throughout the training process (6–7 wk), expression of genes involved in mitochondrial and metabolic regulation appears to decline steadily, indicating an attenuated gene expression response to consecutive acute bouts (14, 38, 47). Similarly, we have recently shown that short-term RE training (12 wk) can attenuate the expression of genes associated with immune responses, although some genes in the same ontology responded in the opposite way (22). Although it is clear that exercise training can attenuate gene expression, this observation is contradictory to the hypothesis in which each subsequent bout is believed to result in an enhanced acute transcriptional response. Thus a better understanding of targeted gene expression within specific biological processes will enhance the interpretation of gene expression dynamics during RE training-induced skeletal remodeling and hypertrophy.

Exercise training-associated phenotypes develop progressively. Because the different biological processes associated with muscle hypertrophy occur at different stages during the training period, the generalization that training causes a desensitization of gene expression seems counterintuitive and misleading. To further clarify whether acute RE-induced gene expression is attenuated by training, we determined the expression pattern of a focused group of genes associated with various processes involved in the development of skeletal muscle hypertrophy. We hypothesized that RE training will differentially alter acute RE-induced gene expression even for genes involved in the same biological process. We found that during skeletal muscle remodeling and subsequent hypertrophy, acute gene expression was multidimensional and ontology-independent (i.e., genes involved in the same biological process displayed a different sensitivity to training). Therefore, we conclude that changes in mRNA levels do not necessarily reflect the actual adaptive response(s) taking place during the training process, which challenges the use of gene expression as a marker of exercise-induced adaptations.

Address for reprint requests and other correspondence: G. A. Nader, Karolinska Institute, Dept. of Physiology and Pharmacology, von Eulers väg 8, Stockholm, Sweden (e-mail: gustavo.nader@ki.se).

http://www.jappl.org 8750-7587/14 Copyright © 2014 the American Physiological Society 693
MATERIALS AND METHODS

Subjects. Thirteen subjects (seven men, six women) participated in the present study. Their mean age was 24 ± 1.4 yr. Inclusion criteria for the study was defined by 1) age between 18 and 40 yr; 2) absence of chronic disease; 3) no prior RE training history; and 4) no current use of medications or dietary supplements that may affect muscle mass (i.e., protein supplements, creatine, steroids, contraceptives, etc.). Subjects were instructed to maintain their normal diet for the duration of the study to prevent diet-related fluctuation in body weight. All participants gave informed consent, and all procedures were approved by the local ethical committee.

RE training and group designation. RE training consisted of a supervised, 12-wk progressive weight-lifting program of the upper arm 2 days per week as previously described (22). Subjects were randomly allocated to one of two groups: the training + acute exercise group (n = 7) trained the nondominant (ND) arm and used the dominant (D) arm as a nonexercise control. The ND (trained) arm was subjected to an acute bout of RE 7 days after the last exercise bout of the 12-wk training program. The acute exercise group (n = 6) received the same treatment as the training + acute group in the ND arm and then performed acute RE in both ND and D arms 1 wk after the completion of the training program. The acute bout was performed at the same relative intensity as the last RE bout [i.e., 80% of 1 repetition maximum (RM)]. Because the goal of the present study was to determine the effect of RE training on gene expression following acute RE, we designated the D arms of those in the training + acute group as control (i.e., no exercise), the ND arm of both groups as training + acute RE, and the D of the acute group as acute RE. This design allowed us to dissect the changes in gene expression following acute RE in the trained and untrained states and to minimize the number of biopsies taken on each subject.

Pre- and postexercise testing. Body weight was measured prior to and following 12 wk of training to ensure that subjects did not experience fluctuations in body weight. Magnetic resonance imaging (MRI) was used to assess changes in whole muscle cross-sectional area (CSA). Pretraining MRI scans were performed before or 48 h after 1 RM testing. Posttraining MRIs were performed 48–96 h after the last training session to avoid any temporary fluid shifts that would influence the results. To ensure accurate and reliable measurements, six slices from each image were analyzed using the metaphyseal-diaphyseal junction landmark, making sure the same regions were measured pre- and posttraining. Cortical bone, subcutaneous fat, and skeletal muscle were isolated using image signal intensity differences between tissues, and once the region of interest was segmented, total volume was determined for the six evaluated slices. Repeatability and reliability of Rapidia® volume measurements were verified using a phantom of known volume. Dynamic strength was tested using the 1 RM weight for the elbow flexors on a standard preacher bench prior to and following 12 wk of RE training. Two warmup sets were completed at 50% and 75% of the predicted 1 RM for 8 repetitions and 5 repetitions, respectively. Single attempts were performed until one single repetition with full range of motion was completed. Dynamic strength gain was determined by calculating the percent difference between pretraining and posttraining 1 RM strength.

Muscle biopsies. Biopsies were obtained bilaterally from the bicep brachii using the percutaneous needle biopsy technique. Four hours after the completion of the acute bout of RE, local anesthesia (3–5 ML of lidocaine HCl) was applied, and a 1-cm incision in skin and fascia was made. Biopsies were obtained with the aid of suction. Samples were cleaned from visible fat and connective tissue, dry blotted, immediately frozen in liquid nitrogen, and stored at −80°C for subsequent analyses.

Protein extraction and Western blotting. Muscle samples were homogenized in ice-cold lysis buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 2.5 mM EGTA, 1% Triton X-100, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium fluoride, and 0.1 mM sodium orthovanadate) supplemented with one tablet of Complete mini protease inhibitor cocktail and Phostop (Roche Diagnostics, Indianapolis, IN) using a 5-mm generator coupled to a Polytron tissue disruptor (Kinematica, Kriens, Switzerland). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Crude homogenates were rotated on a spinning wheel for 30 min at 4°C, spun down, and supernatants were transferred to new tubes. Protein concentration was determined using the DC protein assay (Biorad, Hercules, CA), and the lysate was diluted with lysis buffer before mixing 1:1 with 2× Laemmli buffer (Biorad) containing 5% β-mercaptoethanol. Samples were boiled at 95°C for 10 min and immediately cooled on ice before being stored at −20°C until further use. Samples containing equal amounts of protein (50 μg) were separated by SDS-PAGE on 4–12% polyacrylamide Criterion Gradient gels (Biorad) and transferred to polyvinylidene difluoride membranes (Biorad) activated in 100% methanol. Western blotting was performed using standard techniques, membranes were washed in Tris-buffered saline with 0.1% Tween (TBS-T 0.1%), and blocked in a protein containing buffer (TBS-T 0.1% + 5% milk). Primary and secondary antibodies were diluted in either 5% nonfat dry milk or 5% BSA in TBS-T 0.1%. Primary antibodies (pS6 and PO4-pS6) were purchased from Cell Signaling (Beverly, MA), except for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was from Santa Cruz Biotechnology (Santa Cruz, CA).

RNA extraction, cDNA synthesis, and qRT-PCR. Total RNA was extracted using TRIzol by standard procedures. RNA was subsequently cleaned through RNeasy mini columns and DNase treated, quantified using a Nanodrop, and integrity assessed by agarose gel electrophoresis. RNA was reverse-transcribed with the VILO cDNA synthesis kit (Invitrogen, Carlsbad, CA) using 1 μg of RNA according to the manufacturer’s recommendations. Quantitative reverse polymerase chain reaction (qRT-PCR) was performed using GoTaq PCR mix (Promega, Fitchburg, WI) on the real-time PCR detection system CFX384 (Biorad). Primers were designed using Primer 3 and verified in the National Center for Biotechnology Information’s Primer-BLAST. Primer sequences will be made available upon request. For each primer pair, we performed a melting curve analysis and agarose electrophoresis of the amplon to ensure amplification of a single product of the calculated size. Each primer pair was optimized on a heat gradient to ensure amplification efficiency around 95–100%. All reactions were run on clear-well, hard-shell plates (Biorad) and run in triplicate for each target gene. Relative expression levels were obtained by normalization to GAPDH by the comparative CT (2-ΔΔCT) method in the Bio-Rad CFX Manager (version 3.0) software.

Statistical analysis. Differences in strength and CSA are reported as means ± SE. Comparisons were made using a two-way ANOVA with factors gender and training status followed by the Bonferroni post hoc test (significance level set at P < 0.05). All gene expression values are reported as means ± SE. Differences between groups for qRT-PCR data and Western blotting were determined using a one-way ANOVA, and significance between groups was established using the Newman-Keuls multiple comparison test (P < 0.05).

RESULTS

Skeletal muscle hypertrophy and strength gains. Twelve weeks of RE training resulted in morphological and functional adaptations in both men and women. The mean increase in skeletal muscle CSA was 13.8 ± 1.81% (men 14.8 ± 2.8%, women 12.6 ± 2.4%; P < 0.05). As expected, muscle hypertrophy was accompanied by an increase in dynamic strength of the trained (ND) arm in both men (48.2 ± 5.4%; P < 0.01) and women (72.1 ± 9.1%; P < 0.01) without significant changes in the untrained (D) arm (Fig. 1). Strength changes as determined by 1 RM testing in men were 12 ± 1.4 kg vs. 17.6 ± 1.8 kg for the ND arm and 13 ± 1.4 kg vs. 13.6 ± 1.4 kg for the D arm.
arm, and in women were 6.8 ± 0.8 kg vs. 11.5 ± 1.1 kg for the ND arm and 7 ± 0.9 kg vs. 6.8 ± 0.8 kg for the D arm.

Training attenuated acute exercise-induced intracellular signaling. To verify whether training modified the acute anabolic signaling response to RE, we determined changes in protein phosphorylation following an acute bout of RE in the untrained and trained states. Acute RE increased the PO4-rpS6/rpS6 ratio (approximately 16-fold; P < 0.05) in previously untrained skeletal muscle. However, following 12 wk of RE training, the acute intracellular signaling response based on this marker was attenuated (~50%; NS) from acute RE (Fig. 2).

Training modulated the acute response to RE. In the present study we chose a selected set of transcripts induced in both men and women that are involved in various adaptive processes important for skeletal muscle hypertrophy. We chose four to six targets per biological process to determine whether genes involved in the same biological process respond differentially to acute RE in the naïve and trained states. Furthermore, gene expression following RE showed a heterochronic pattern [i.e., some genes are only expressed in the trained state (Fig. 3)].

Training attenuated the acute induction of growth genes. Acute RE significantly induced the expression of genes involved in skeletal muscle hypertrophy and other growth processes. Ribosomal DNA transcription rates (45S pre-rRNA) increased 2.2-fold (P < 0.001); c-Myc and ATF3 were induced 52-fold and 155-fold, respectively (P < 0.001) by acute RE. CTGF was also significantly induced by acute RE (12-fold; P < 0.001). In the trained state, however, acute induction of these genes was less responsive, rDNA transcription was significantly attenuated (~40%; P < 0.001), and c-Myc and ATF3, although modulated by training, remained slightly above control levels. Expression of CTGF was completely attenuated by training (Fig. 4).

Training suppressed the expression of negative regulators of muscle mass and protein degradation genes. Acute RE training resulted in a marked reduction in myostatin mRNA levels (~21%; NS), which was further reduced in the trained state (~73% and ~66% from control and acute RE, respectively; P < 0.001). Tripartite motif (TRIM)32 mRNA levels were lower than control in response to acute RE (~31.5%; P < 0.05), and training did not modify this response. Interestingly, expression of the 97-kDa Valosin Containing Protein (p97/VCP) mRNA increased acutely (1.4-fold; P < 0.05) in naïve skeletal muscle, but the response to acute RE in the trained state was significantly (~45%; P < 0.05) attenuated (Fig. 5).

Training resulted in a multiphasic expression of angiogenesis and metabolism genes. Angiogenic mRNAs responded to RE in a multiphasic manner. Acute RE induced CYR61 47-fold (P < 0.001) from control, but this response was strongly...
attenuated by training (−95%; \( P < 0.001 \)). VEGF displayed a different pattern of expression in that it was induced by acute RE (NS), and the response was maintained in the trained state (2-fold; \( P < 0.01 \)). ANGPTL2 responded acutely (1.7-fold; NS) and was significantly enhanced by training (2.5-fold; \( P < 0.01 \)), whereas ANGPT2 decreased significantly in the trained state (−38%; \( P < 0.05 \)) (Fig. 6). HBEGF (19-fold; \( P < 0.001 \)) and INSIG-1 (3.5-fold; \( P < 0.05 \)) showed the strongest induction following acute RE with a significant attenuation in the trained state (−69%; \( P < 0.001 \) and −25%; \( P < 0.05 \) respectively). Interestingly, PDK4 did not increase with exercise following acute RE in either naive or trained muscle. IL6R expression was induced acutely (6-fold; \( P < 0.001 \)) regardless of the training state of the muscle. NR4A1 and NR4A3 increased following acute RE, by 9-fold and 36-fold, respectively (\( P < 0.001 \)), and this response was not attenuated by RE training (Fig. 7).

A notable exception to these transcriptional patterns of gene expression was PGC1α. We initially determined PGC1α mRNA levels using primers that do not discriminate among isoforms and found that acute RE in the trained state resulted in a 1.9-fold \( (P < 0.05) \) increase compared with acute RE in untrained muscle and control (data not shown). However, due to the complex splicing variants arising from this gene, we also determined the effects of RE training on the expression of the various PGC1α isoforms. Analysis of the splice variants show that PGC1α1 was reduced, although not significantly, by acute RE regardless of the training state. PGC1α2 and PGC1α3 had similar induction patterns following acute RE (22-fold to 27-fold, respectively; \( P < 0.05 \)) with the magnitude of the response exacerbated by training (2-fold to 3-fold; \( P < 0.05 \)). PGC1α4, however, was not responsive to acute RE, but was significantly induced in the trained state (1.9-fold; \( P < 0.05 \)) (Fig. 8).

Table 1 shows a number of genes studied that were not modulated by acute RE in either the untrained or trained states.

**DISCUSSION**

The goal of the present study was to determine whether RE training could modulate exercise-induced mRNAs associated with muscle adaptation. Because relative changes in muscle strength and hypertrophy following training were similar in men and women, we specifically focused on a subset of genes commonly induced in both genders. We studied expression following an acute bout of RE in the untrained and trained states. Following 12 wk of RE training, muscle hypertrophy was apparent in both men and
women, and this was accompanied by changes in dynamic strength. Because muscle hypertrophy would have continued concomitantly with the training program in both men and women at least up to 16 wk (12), we consider our subjects to have been undergoing active muscle remodeling during the duration of the study.

The first issue we addressed was whether training resulted in attenuated acute exercise responses. We determined the acute response of rpS6 phosphorylation as an indicator of acute hypertrophic stimulation and found that training indeed ameliorated the acute anabolic signaling response to RE. A recent study in rodents (35) reported similar findings, which allow us to conclude that RE training attenuates acute signaling responses to RE. This observation is also consistent with the attenuated response of nonmyofibrillar protein synthesis previously described in humans following 8 wk of RE training (27). Based on this evidence, together with previous knowledge indicating an attenuated response of gene expression following RE training (22), we asked whether the attenuated response to exercise is a general phenomenon or whether it is gene specific.

One set of mRNAs studied in the present study was from genes involved in cellular hypertrophy. All four targets displayed a similar pattern, in which the acute RE induction was attenuated following RE training. Clearly, this response was inversely correlated with the occurrence of the hypertrophic phenotype (i.e., 12 wk later). During early stages of RE training (~4 wk), strength performance is mainly determined by neural factors (34). Although the morphological changes observed following the commencement of training can be detected as early as 3 wk into the training program (49), this is still significantly later than the first acute bout. Although our sampling time course does not allow for such fine temporal resolution, our findings on 45S pre-rRNA, c-Myc, and ATF3 transcription are similar to what occurs in the rodent functional overload (54) and cardiac pressure overload (29) models. Furthermore, CTGF, which is important for connective tissue growth/remodeling, displayed a similar pattern. Thus acute RE can induce a significant early molecular growth response of muscle and connective tissue that is attenuated with RE training.

Consistent with previous data (25), changes in myostatin mRNA were slightly below control levels following acute RE, but strongly suppressed after RE training, indicating that muscle catabolism/growth arrest can be modulated by RE training in a time-dependent manner. Although muscle atrophy is seen as the opposite of hypertrophy, protein degradation ensures that damaged or misfolded proteins during exercise are adequately removed from the cell and is therefore an important aspect of the remodeling process. We investigated the dynam-

![Fig. 5. Changes in mRNA levels of growth arrest and proteolysis genes in control and exercised muscle. Relative expression of myostatin, TRIM32, and p97/VCP in control (white bar, n = 6), acute RE (gray bar, n = 7), and 12 wk RE training + acute RE (black bar, n = 13). *Significantly different from control; P < 0.05. †Significantly different from acute RE; P < 0.05. Transcript levels were normalized to GAPDH and comparisons between groups were test using a one-way ANOVA followed by the Newman-Keuls post hoc test.](image)

![Fig. 6. Changes in mRNA levels of angiogenesis genes in control and exercised muscle. Relative expression of CYR61, VEGF, ANGPTL2, and ANGPT2 in control (white bar, n = 6), acute RE (gray bar, n = 7), and 12 wk RE training + acute RE (black bar, n = 13). *Significantly different from control; P < 0.05. †Significantly different from acute RE; P < 0.05. Transcript levels were normalized to GAPDH and comparisons between groups were test using a one-way ANOVA followed by the Newman-Keuls post hoc test.](image)
ics of several mRNAs involved in both ubiquitin proteasome and autophagy-mediated protein degradation systems. Interestingly, although most of the mRNAs studied did not change significantly with acute RE at this time point (Table 1), TRIM32 and p97/VCP were modulated by acute RE, albeit in opposite directions, although they were similarly and significantly suppressed following RE training. TRIM32 is involved in thin-filament ubiquitylation and sarcomere disassembly, specifically by targeting desmin and the Z-bands to disrupt sarcomeric integrity (11). p97/VCP is an ATPase that serves as a cofactor for 26S proteasome function ubiquitin-dependent protein degradation (4). Mutations leading to p97/VCP loss of function result in protein aggregates, and this is believed to cause inclusion body myopathy (53). These findings, together with the fact that a reduction in TRIM32 levels can prevent the loss of both desmin and other thin filament proteins (11), may explain how RE training ameliorates the response of these mRNAs to acute exercise. Furthermore, because RE training reduces protein turnover (52), one possible functional interpretation of the reduced expression of TRIM32, p97/VCP, and myostatin could be the reduction in muscle protein degradation and the maintenance of sarcomeric integrity typical of repeated bouts of RE. These findings may shed light into the mechanisms by which muscle protein turnover accommodates to acute RE in the trained state.

Exercise has long been recognized as a promoter of arterial health, but this effect has been mainly associated with EE (48).

Fig. 7. Changes in mRNA levels of substrate metabolism genes in control and exercised muscle. Relative expression of HBEGF, INSIG-1, PDK4, IL6R, NR4A1, and NR4A3 in control (white bar, n = 6), acute RE (gray bar, n = 7), and 12 wk RE training + acute RE (black bar, n = 13). *Significantly different from control; P < 0.05. †Significantly different from acute RE; P < 0.05. Transcript levels were normalized to GAPDH and comparisons between groups were test using a one-way ANOVA followed by the Newman-Keuls post hoc test.

Fig. 8. Changes in mRNA levels of PGC1α splice isoforms in control and exercised muscle. Relative expression of PGC1α1, PGC1α2, PGC1α3, and PGC1α4 in control (white bar, n = 6), acute RE (gray bar, n = 7), and 12 wk RE training + acute RE (black bar, n = 11). *Significantly different from control; P < 0.05. †Significantly different from acute RE; P < 0.05. Transcript levels were normalized to GAPDH and comparisons between groups were test using a one-way ANOVA followed by the Newman-Keuls post hoc test.
In addition to inducing muscle hypertrophy, the training protocol employed in the present study also resulted in vascular remodeling as determined by ~6% increase in brachial artery diameter (56). This is consistent with previous findings indicating that RE-trained men have higher overall endothelial function and venous compliance than untrained controls (26, 43). To further understand how RE training may induce vascular remodeling, we investigated the expression of key genes involved in angiogenesis. CYR61, a strong promoter of angiogenesis (2, 28), was highly induced by acute RE, but this response was completely blunted by RE training. Interestingly, VEGF was induced acutely regardless of the training state (although only significantly in the trained state), and both ANGPTL2 and ANGPT2 were modulated by RE training in the trained state but in opposite directions. Although the precise role these factors play in RE-induced angiogenesis is not well understood, our results suggest that their dynamic expression pattern may reflect possible distinct roles in vascular remodeling during hypertrophy. Further support for this interpretation can be drawn from previous studies demonstrating positive vascular adaptations to RE in human and animal models (i.e., fiber hypertrophy is accompanied by an increase in capillary number). For example, in humans, 12 wk of RE training resulted in capillary changes proportional to muscle fiber hypertrophy (33), and in rodents, mechanical loading increases in muscle fiber CSA was accompanied by increased capillary neoformation in a time-dependent manner (17, 41). Even though it is difficult to directly compare the magnitude of the changes in hypertrophy between other models and our cohort, what becomes clear from our data is that changes in proangiogenic mRNAs occur at different time points during muscle hypertrophy and are likely involved in the initiation and progression of the vascular remodeling that accompanies skeletal muscle hypertrophy.

The cellular and molecular muscle metabolic adaptations consequent to RE training remain largely understudied compared with growth-related mechanisms. Although previous research has been indicative of moderate to negligible metabolic adaptations (51), recent studies have challenged these findings in support of enhanced metabolic plasticity with RE training (23, 46, 50). To further expand the knowledge about how RE training results in metabolic adaptations, we studied a set of genes with known roles in metabolic regulation. We found that consistent with the angiogenic response, the expression pattern of some of these genes was also dependent on the training state. HBEGF and IL6R are both involved in muscle glucose uptake, and therefore, their enhanced expression may reflect one possible mechanism by which RE training improves glycemic control (1, 19). Despite their similar function, HBEGF responded to acute RE but its expression was attenuated in the trained state. IL6R, however, was responsive to acute RE regardless of the training state, again highlighting the temporal mismatch of gene expression among targets involved in a similar process (i.e., glucose uptake). PDK4, previously shown to respond to acute RE, did not significantly change in the biceps regardless of the training state. This is surprising because the time point studied herein and the primers used to detect its mRNA were exactly the same as previously reported (55). One key difference may be the muscle sampled; whereas we studied the biceps, others reported changes in the vastus lateralis (10, 55). Thus muscle sampling differences may exist that preclude a direct comparison of the acute expression of certain genes in response to exercise. INSIG-1, a key regulator of lipogenesis, was induced by acute RE and like HBEGF, the response was attenuated with training. Because of its role in preventing lipid accumulation, one possible function of INSIG-1 in RE training-mediated adaptation could be an initial reduction of lipogenesis, was induced by acute RE and like HBEGF, the response was attenuated with training. Because of its role in preventing lipid accumulation, one possible function of INSIG-1 in RE training-mediated adaptation could be an initial reduction of lipogenesis. As training-induced metabolic adaptation ensues, it may facilitate the accommodation of lipid storage/usage in the trained state (15, 46, 50). Both nuclear orphan receptors NR4A1 and NR4A3 were induced by acute RE in a similar magnitude regardless of the training state. Like the proangiogenic response, we interpret the changes in these mRNAs as facilitating different aspects of muscle metabolism. Studies in genetically modified mice demonstrated that the NR4 receptors play key roles in the modulation of lipolysis, enhanced insulin action, increase in oxidative capacity, and fatigue resistance (6, 7, 18, 32, 36). Even though the precise function in human skeletal muscle is unknown, the NR4 gene expression responses obtained in the present study favor the idea that the
NR4s may play a role in RE-induced muscle metabolic adaptations both acutely and during training. Thus the expression of genes involved in metabolic regulation following acute RE is heterochronic and highly sensitive to the training state of the muscle.

We also determined the dynamics in PGC1α because this gene is known to regulate several aspects of muscle metabolism (42) and is rapidly induced by acute RE and EE (40). Given the recent discovery of the isoform diversity of PGC1α, we defined whether the newly described splice variants showed an expression pattern consistent with previous PGC1α data and the occurrence of muscle hypertrophy. Acute RE resulted in a reduction in PGC1α mRNA even in the trained state, and whereas the α2 and α3 isoforms responded to acute exercise, the response in the trained state was superior to untrained muscle. Notably, the α4 isoform was responsive only in the trained state. At present, the role of the different isoforms is rather inconclusive except for the α4 isoform, which has been implicated in the development of muscle hypertrophy. Overexpression of α4 can induce several anabolic genes in cultured myotubes, and mice overexpressing this isoform are resistant to muscle atrophy either during inactivity or cachexia (45). However, despite its apparent function in muscle hypertrophy, a recent study demonstrated that mice lacking the PGC1α gene can undergo work-induced hypertrophy (37). Naturally, these data do not permit a proper interpretation of the timing of this isoform’s expression in human skeletal muscle. Furthermore, because of technical issues (i.e., mRNA target sequence) and the lack of previous data on the expression of the isoforms, it is difficult to compare our results with those previously reported for PGC1α in response to acute RE. Nevertheless, it is clear that the isoform diversity of PGC1α expression is highly divergent, and this observation highlights the need to better understand the role of PGC1α in exercise-induced skeletal muscle adaptation.

Given the large diversity in study designs and muscles sampled, it is difficult to establish direct comparisons between the present and previously published results. However, one important aspect of the present study is that for some genes common responses can be observed, indicating despite logistical differences, a conserved response to RE exists. One major limitation, however, is the lack of protein analyses of the corresponding mRNAs studied. This does not permit the interpretation of protein steady states during the adaptive response or correlation with changes in the cognate mRNAs. Nevertheless, it is clear from our results that gene expression in response to acute RE following a short training period is subjected to regulatory mechanisms that may dictate when genes are expressed along the training process. This in turn may establish a hierarchy of genomic responses involved in initiating and/or maintaining training-induced adaptations. In the present study, we examined the dynamic interaction between acute RE-induced gene expression and RE training. We found that following 12 wk of supervised training, changes in mRNA levels were multidimensional (i.e., some genes responded to acute RE, but some others did not, and some showed either an enhanced or suppressed response following training, whereas some responded only in the trained state). Despite its limitations, our data serve as a starting point for the design of studies aimed at determining how the changing milieu of the trained muscle modulates gene expression. A number of attractive possibilities may explain this phenomenon; for instance, changes in DNA methylation (3) may silence/activate gene expression, and this may vary according to the training state. Likewise, changes in mRNA turnover may be affected by accumulation of regulatory or splicing factors (21, 30). Regardless of the mechanism, our results shed light into the multidimensional nature of gene expression during skeletal muscle remodeling. They also provide further evidence indicating that the attenuation of acute responses to training is selective, which warrants further investigation into how exercise induces gene expression in the various training states. Finally, because the genes studied are involved in various key processes regulating skeletal muscle adaptations to RE, we conclude that gene expression following training is highly sensitive to the training state, and does not necessarily reflect the actual adaptive response taking place during the training process.

ACKNOWLEDGMENTS

We thank Dr. Jorge Ruas for the kind gift of the PGC1α isoform primers.

GRANTS

This work was supported by grants from the Swedish Research Council, Centrum för Idrottsforskning, Kung Gustaf V’s 80 Arsfond, and Diabetesförbundet.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


