Effect of High-Frequency Resistance Exercise on Adaptive Responses in Skeletal Muscle

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ABSTRACT

COFFEY, V. G., D. W. REEDER, G. I. LANCASTER, W. K. YEO, M. A. FEBBRAIO, B. B. YASPELKIS 1112, DONALD W. REEDER, GRAEME I. LANCASTER, WEE KIAN YEO, MARK A. FEBBRAIO, BEN B. YASPELKIS 31, and JOHN A. HAWLEY. Effect of High-Frequency Resistance Exercise on Adaptive Responses in Skeletal Muscle. Med. Sci. Sports Exerc., Vol. 39, No. 12, pp. 2135–2144, 2007. Purpose: Regulation of skeletal muscle mass is highly dependent on contractile loading. The purpose of this study was to examine changes in growth factor and inflammatory pathways following high-frequency resistance training. Methods: Using a novel design in which male Sprague-Dawley rats undertook a “stacked” resistance training protocol designed to generate a summation of transient exercise-induced signaling responses (four bouts of three sets x 10 repetitions of squat exercise, separated by 3 h of recovery), we determined the effects of high training frequency on signaling pathways and transcriptional activity regulating muscle mass. Results: The stacked training regimen resulted in acute suppression of insulin-like growth factor 1 mRNA abundance (P < 0.05) and Akt73 phosphorylation (P < 0.05), an effect that persisted 48 h after the final training bout. Conversely, stacked training elicited a coordinated increase in the expression of tumor necrosis factor alpha, inhibitor kappas B kinase alpha/beta activity (P < 0.05), and p38 mitogen-activated protein kinase phosphorylation (P < 0.05) at 3 h after each training bout. In addition, the stacked series of resistance exercise bouts induced an increase in p70 S6 kinase31 phosphorylation 3 h after bouts x3 and x4, independent of the phosphorylation state of Akt. Conclusions: Our results indicate that high resistance training frequency extends the transient activation of inflammatory pathways and transcriptional activity regulating skeletal muscle mass in vivo. Key Words: CELL SIGNALING, mRNA, RESISTANCE TRAINING, RECOVERY

Habitual exercise training generates a multitude of integrated adaptive responses in skeletal muscle, and the adaptation to repeated exercise stimuli is a consequence of the inherent need to maintain homeostasis and minimize cellular disruptions/disturbances during subsequent exercise bouts. Regular exercise training of sufficient overload initiates a cascade of events in muscle that upregulates gene expression and results in modification of adaptive responses (12,26,33). The signaling pathways that initiate adaptive processes in skeletal muscle constitute a complex network of kinases and phosphatases that are often subject to a high degree of feedback regulation, cross-talk, and transient activation. The key components that stimulate the adaptation response are the volume, intensity, and frequency of training sessions, with the sum of these inputs termed the training stimulus or overload. Although there is obviously a training stimulus beyond which any additional load or stimulus does not induce further desired adaptation, the control mechanisms for the adaptive process require regular periods of overload regardless of the exercise mode. However, an imbalance between training frequency and subsequent recovery may give rise to an accumulation of training stress that results in a suboptimal adaptation response in skeletal muscle, termed overtraining. Therefore, the frequency of overload is important in defining the training stimulus, with adequate recovery required to ensure optimal muscle adaptation.

Resistance training is recognized as the primary mode of training for increasing muscle strength and power (20). Resistance exercise consists of large muscle groups undertaking short bouts of high-load, low-repetition exercise to increase the cross-sectional area and force-generating capacity of the muscle. Although our current understanding of the signal transduction pathways controlling skeletal muscle hypertrophy and atrophy in response to resistance training are incompletely understood, a growing body of evidence identifies several key proteins that are pivotal to the regulation of muscle mass. For example, activation of insulin-like growth factor 1 (IGF-1) signaling and Akt/mammalian target of rapamycin/p70 S6 kinase is sufficient
to induce skeletal muscle hypertrophy (5), and this signaling cascade can also block the transcriptional upregulation of key mediators of skeletal muscle atrophy (29), the ubiquitin-ligases muscle atrophy F box (MAFbx, also called Atrogin), and muscle RING finger 1 (Murf1). Activation of the nuclear factor κB (NF-κB) transcription pathway, by catabolic factors such as tumor necrosis factor alpha (TNFα) and inhibitor NF-κB kinase, is sufficient to induce skeletal muscle atrophy, in part via NF-κB-mediated upregulation of Murf1 (7). The aim of this study was to determine the acute response of the IGF-1 and TNFα pathways to an excessive accumulation of training stress. Here, we used a novel “stacking” resistance training protocol designed to generate a summation of transient exercise-induced growth factor/inflammatory signaling and transcriptional responses. We hypothesized that when resistance training sessions were performed with short (i.e., 3 h) recovery periods, the transient response of key signaling pathways implicated in the regulation of muscle mass would be extended and additive, exacerbating the molecular responses to acute, exercise-induced overtraining.

METHODS

Experimental Design

Forty-two male Sprague–Dawley rats about 6 months of age (mass 351 ± 18 g) were obtained from Harlan (San Diego, CA) and housed four per cage in an environment maintained at ~21°C with an artificial 12:12 h light–dark cycle. All animals received a standard Chow diet (63% carbohydrate, 17% fat, and 20% protein; no. 112386, Dyets, Bethlehem, PA) for the duration of the study and had ad libitum access to food and water. Animals were assigned to either control (N = 6) or stacked resistance training (N = 36) groups. The resistance exercise group was further subdivided into stacked training groups that completed either one, two, three, or four exercise sessions, each separated by 3-h recovery periods, performed on the same day (x1, x2, x3, or x4; N = 6 per group), with each group being sacrificed 3 h after exercise. Two additional groups of animals also completed all four exercise sessions in a single day and were sacrificed 24 and 48 h (N = 6 per group) after their last training bout. Each session/bout comprised three sets of 10 repetitions of squat exercise, with 3 min of recovery between sets (described subsequently). All experimental and training procedures were approved by the institutional animal care and use committee at California State University, Northridge and conformed to the guidelines for the use of laboratory animals published by the U.S. Department of Health and Human Resources.

Resistance Training Protocol

One-repetition maximum (IRM) was determined for all animals a minimum of 5 d before commencement of a training period. The IRM established the physical load that could be lifted once but not a second time, with the same squat training equipment that was used during all training sessions. Mean IRM for all animals was 989 ± 63 g. All subsequent resistance training protocols were performed at 75% of IRM. The core components of the training protocol and apparatus used in the present study have been described in detail previously (26). Briefly, the apparatus consisted of two 45-cm vertical metal rods 0.5 cm in diameter, set 31 cm apart on a 15 x 20-cm stainless steel metal base securely inserted into a 2.54-cm-thick wood platform. A 33 x 2.54-cm wood crossbeam was outfitted with two brass sleeves and placed on the two vertical rods, allowing for uninhibited vertical movement. An aluminum holder, molded to accommodate the rats, was attached to the center of the crossbeam at a 90° angle relative to the base. Animals were strapped into a nylon vest and attached to the aluminum holder with Velcro straps before being placed in a squat position, using safety stops on each rod to support the load. Two 5-cm metal pegs attached vertically on opposite ends of the crossbeam were used to mount calibrated miniature weight plates. A brief, low-voltage electrical stimulus (10 V, 0.3-s duration) was delivered by manually pressing a switch that allowed current to flow through an electrode attached to the tail of the animals to initiate each repetition. After each repetition, the animals were repositioned on the apparatus such that their legs were beneath the torso. A repetition was initiated as soon as practicable after each prior repetition (i.e., cessation of movement and repositioning; mean interval z 3 s) and repeated until 10 repetitions were completed. The animals were allowed to rest for 3 min between each set and were removed from the apparatus and returned to their cage during the recovery period. Group sacrifice (N = 6) was conducted 3 h after every resistance training session, and 24 and 48 h after the completion of the training protocol. Animals were sacrificed via cervical dislocation and prepared for hind limb muscle collection. We chose to extract white quadriceps muscle because of the mechanics of training and major contribution of fast-twitch fibers to the hypertrophy response with resistance training and defining the strength phenotype (14,20). Portions of white quadriceps were surgically removed from the left and right hind limbs in sequence and freeze-clamped in liquid N2. Muscle samples were stored at −80°C until subsequent analysis.

Analytical Procedures

Muscle glycogen concentration. Muscle samples (~40–50 mg) were freeze-dried with visible connective tissue removed during powdering. Aliquots (~3 mg) of freeze-dried muscle were extracted with 250 μL of 2 M hydrochloric acid, incubated at 100°C for 2 h, and then neutralized with 750 μL of 0.67 M sodium hydroxide for subsequent determination of glycogen concentration via enzymatic analyses (50 mM Tris, 25 mM HCl, 1 mM MgCl2, 0.5 mM DTT, 0.3 mM ATP, 0.05 mM NADP,
washed with TBST and incubated with an appropriate primary antibodies overnight at 4°C. Membranes were incubated with appropriate secondary antibodies for 60 min. Proteins were visualized by chemiluminescence and quantified by densitometry. A sample from each different training group was run on the same gel, and the amount of protein from the densitometric quantification is expressed as arbitrary units.

Measurement of mRNA

RNA was isolated, using a modification of the acid guanidinium thiocyanate-phenol-chloroform extraction for total RNA method of Chomczynski and Sacchi (10). Briefly, approximately 30 mg of frozen muscle was homogenized in 1 mL of Trizol reagent (Invitrogen, Australia), with 200 μL of chloroform subsequently added, and the samples were incubated for 5 min at room temperature. Samples were then centrifuged at 12,000g for 15 min at 4°C. The upper aqueous phase was transferred to a sterile tube before adding 500 μL of isopropanol. After 10 min of incubation at room temperature, samples were centrifuged at 12,000g for 10 min at 4°C. Supernatant was removed, leaving the RNA pellet, which was washed in 500 μL of 75% ethanol and centrifuged at 7500g for 5 min at 4°C. The ethanol was then removed, and the pellet was allowed to dry and then resuspended in 10 μL of DEPC-treated water. The total RNA content was established, measuring absorbance readings at 260 and 280 nm. For each sample, 0.1 μg of RNA was reverse transcribed in a 10-μL reaction, using a Taqman Reverse Transcription Reagents kit (N8080234, Applied Biosystems), including appropriate RT-negative controls as described elsewhere (19). Reverse transcription reactions were performed using a 2700 Peltier Thermal Cycler (Bio-Rad, Hercules, CA) at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min, and samples were stored at −20°C until analysis.

Real-Time Polymerase Chain Reaction

Preoptimized commercial PCR probes and primers (Applied Biosystems) were obtained for IGF-I (assay no. Rn00710306), muscle F-box only protein 32 (MAFbx; Rn00591730), and tripartite motif containing 63/muscle RING finger protein (MuRF1; Rn00590197). We quantitated mRNA abundance using multiplex comparative critical thresholds (Ct) with ribosomal 18S as a reference gene (Bio-Rad i Cycler IQ, Hercules, CA). PCR was carried out in duplicate in 25-μL reactions of (1×) BioRad iQ Supermix (12.5 μL), assay on demand probe/primer (1.25 μL), (0.5×) 18S probe/primer (0.63 μL), DEPC-treated water (9.63 μL) and 1 μL of sample cDNA (10 ng μL⁻¹). cDNA was amplified, using the following conditions: 50°C for 2 min, 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. 18S Ct values were subtracted from the gene of interest Ct values to derive a ΔCt value for each animal. The expression of the gene of interest relative to 18S control was then calculated, using the expression expression ΔΔCt value. The expression of the genes of interest was then evaluated, using the expression 2⁻ΔΔCt, and independent group means were compared in the analysis of variance (ANOVA) model.

Myofibrillar Protein Extraction

Procedures to determine myofibrillar protein concentration were modified from those described elsewhere (13). Briefly, approximately 5 mg of powdered muscle was homogenized in buffer (0.15 NaCl, 0.1% Triton, 0.02 M Tris, 50 μM DTT, 0.1 M EDTA, 1 mM PMSF) and
centrifuged at 1600g for 20 min to produce a myofibrillar pellet. After removal of the supernatant, the pellet was washed in low-salt buffer (100 mM KCl, 5 mM Tris-Cl, 1 mM DTT, pH 7.4) and centrifuged for 5 min at 13000g, repeated twice. The pellet was then washed twice in 70% ethanol solution. After removal of ethanol, the pellet was resuspended in 0.3 N NaOH, and an aliquot was determined to determine the protein content using the Bradford assay (Pierce, Rockford, IL).

Statistical Analysis

Immunoblot phosphorylation and total protein data are expressed in arbitrary units relative to nonexercise controls. Differences between means were determined by a one-way ANOVA with Newman–Keuls post hoc test (SigmaStat for Windows, version 3.11). All values are expressed as means and standard error (SE), with the critical level of significance established at P < 0.05.

RESULTS

Muscle Glycogen

Muscle glycogen content was decreased by about 30% at 3 h after the initial training session (P < 0.05), and it remained below nonexercise control values 3 h after each subsequent exercise bout (~30–37%, P < 0.05, Fig. 1A). Muscle glycogen levels were restored to control values after 24 and 48 h of recovery from the last (x4) bout of resistance exercise.

Signaling Responses

AMPK. As might be expected with the persistent decline in muscle glycogen after each resistance training bout, phosphorylation of AMPK<sup>Thr172</sup> gradually increased approximately 25–40% above control values after multiple (x2–4) training bouts (Fig. 1B). AMPK<sup>Thr172</sup> phosphorylation was highest 3 h after bout 4 compared with 48 h after exercise (~55%, P < 0.05).

Akt/mTOR/forkhead box O1. Phosphorylation of Akt<sup>(Ser473)</sup> was significantly decreased after the second bout of training, and it remained suppressed for 3 h after each bout of resistance exercise for the remainder of this protocol (~35–50%, P < 0.05). In addition, this suppression of Akt persisted for up to 48 h after completion of all four training bouts (Fig. 2A). Conversely, neither phosphorylation of mTOR<sup>Ser2448</sup> (Fig. 2B) nor of forkhead box O1 (FoxO1)<sup>Thr2451</sup> (Fig. 2C) were significantly altered during the training protocol.

p70 S6 kinase/4E-BP1. No difference in 4E-BP1<sup>Ser70</sup> phosphorylation was observed at any time point after resistance training (~15–25%, NS, Fig. 3A). In contrast, phosphorylation of p70 S6 kinase (p70 S6K)<sup>Thr389</sup> was increased above control values at 3 h after each bout of resistance exercise, reaching significance after x3 and x4 bouts of training (~500%, P < 0.05, Fig. 3B). Phosphorylation of p70 S6K had returned to control levels at 24 and 48 h after the final training bout. Further analysis revealed comparable phosphorylation of the p70 S6K<sup>Thr421/ser424</sup> sites, culminating in a significant increase above control after x3 and x4 bouts of training (~600%, P < 0.05, Fig. 3C), returning to baseline after 48 h recovery.

Cytokines and mitogen-activated protein kinase. Resistance exercise resulted in increased expression of TNFα at all postexercise time points (~13–54%) but only attained statistical significance from control values at 3 h after the fourth training bout (P < 0.05, Fig. 4A). However, IKKα/β<sup>Ser180/181</sup> phosphorylation was significantly higher than control after 3 h of recovery after all training bouts (~65–75%, P < 0.05), returning to baseline after 48 h of recovery (Fig. 4B). Similarly, significant increases in p38<sup>Ser180</sup> mitogen-activated protein kinase (MAPK) phosphorylation were consistently observed at 3 h after each bout of exercise—an effect that persisted for 24 h after completion of the training protocol (~67–100%, P < 0.05, Fig. 4C).

mRNA Abundance

IGF-1. Resistance training resulted in a persistent suppression of IGF-1 mRNA abundance at 3 h after
exercise (Fig. 5A). IGF-1 mRNA decreased approximately 80-100% and was different from control values after each of the four bouts of resistance exercise \((P < 0.05)\), returning to baseline after 24 h of recovery. Unexpectedly, the restoration of IGF-1 mRNA abundance was not sustained, and it had decreased below control values at 48 h after completion of the training protocol \((-75\%, \ P < 0.05)\).

MAFBx/MuRF1. MAFBx mRNA was largely unchanged, whereas MuRF mRNA abundance tended to increase 3 h after each training bout (Fig. 5B and C). High-frequency training generated peak mRNA content above control values at 24 h after completion of the training.
protocol, but these changes were not significantly elevated for MAFBx (approximately sevenfold, \( P = 0.06 \)) or MuRF (approximately twofold, NS).

**Myofibrillar Protein Concentration**

Modest increases in protein concentration of the contractile component of skeletal muscle were observed 24 h

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**FIGURE 4**—Total tumor necrosis factor alpha (TNF\(\alpha\)) content (A), inhibitor kappa B kinase \(\beta\) (IkB) phosphorylation (B), and phosphorylated p38\(\alpha\) mitogen-activated protein kinase (MAPK) relative to total (C) after “stacked” resistance training comprising four bouts of 3 x 10 repetitions separated by 3 h of recovery. Repetitions were performed at 75% one-repetition maximum, with 3 min of recovery between sets. White quadriceps muscle was extracted 3 h after every training bout and 24 and 48 h after the final exercise bout (\(\times 4\)) of the protocol. Results are group means (± SE), and data are arbitrary values expressed relative to control. Significant difference (\( P < 0.05 \)) vs * control, # 24 h.

![Graph D](image4)

**FIGURE 5**—Insulin-like growth factor 1 (IGF-1; A), muscle atrophy F box-only protein (MAFBx, also known as Atrogin; B), and muscle RING finger protein (MuRF; C) mRNA abundance after “stacked” resistance training comprising four bouts of 3 x 10 repetitions separated by 3 h of recovery. Repetitions were performed at 75% one-repetition maximum, with 3 min of recovery between sets. White quadriceps muscle was extracted 3 h after every training bout and 24 and 48 h after the final exercise bout (\(\times 4\)) of the protocol. Results are group means (± SE), and data are arbitrary values expressed relative to control. Significant difference (\( P < 0.05 \)) vs * control, # 24 h.

(-13%, 72.7 ± 5.4 mg\(\text{g}^{-1}\), NS) and 48 h (-10%, 70.3 ± 3.5 mg\(\text{g}^{-1}\), NS) after the resistance training protocol, but these changes failed to reach statistical significance (control: 64.1 ± 2.9 mg\(\text{g}^{-1}\)).

**DISCUSSION**

Adaptation to skeletal muscle loading is determined by the volume, intensity, and frequency of contractile activity, and the sum of these inputs initiates various cell processes that modify the regulation of muscle mass. In the present
study, we used a novel stacking protocol in an attempt to identify the effects of altered resistance training frequency on discrete cellular and molecular events after repetitive overload. We hypothesized that high-frequency training sessions would extend the transient response of key signaling pathways, leading to an additive, or stacked, effect, and a concomitant upregulation of signaling pathways and gene transcriptional activity involved in the regulation of skeletal muscle mass. We provide novel evidence to demonstrate that repeated resistance training with short (3 h) recovery periods suppressed IGF-1 mRNA abundance and Akt phosphorylation and exacerbated expression of TNFα, IKK activity and p38 MAPK phosphorylation. In addition, a summation in p70 S6K phosphorylation was observed with repeated overload, independent of the activation states of Akt and mTOR.

As might be expected, high-frequency training bouts resulted in a persistent suppression of muscle glycogen content. It has been proposed that AMPK acts as a "fuel gauge" in muscle cells, switching off ATP-consuming pathways when cellular energy is low while concomitantly turning on alternative pathways for ATP regeneration. Accordingly, the suppressed glycogen content in the working muscles when undertaking the resistance training protocol was associated with a gradual increase in AMPK phosphorylation with each successive bout, representative of a summation in exercise-induced overload (Fig. 1).

A novel finding of this study was the decrease in IGF-1 mRNA abundance in response to the stacking protocol. Moreover, there was a sustained suppression in IGF-1 mRNA and Aktser phosphorylation with repeated bouts of resistance exercise. Skeletal muscle IGF-1 mRNA content has been shown to increase (1,25) and decrease (4,36) in response to contractile overload. Previous work by Haddad and Adams (23) has incorporated two successive bouts of resistance exercise separated by 8, 24, or 48 h. Their results show a reciprocal effect where the increase in IGF-1 mRNA levels were more pronounced after 48 h of recovery and were systematically reduced with shorter recovery periods. Here, we report a persistent decrease in IGF-1 mRNA after multiple bouts of resistance exercise separated by only 3 h recovery periods (Fig. 5). Consequently, it may be reasonable to suggest that a negative correlation exists between IGF-1 gene expression and exercise recovery (< 48 h) where IGF-1 transcriptional activity is impeded or suppressed with a reduced recovery time. Notably, IGF-1 seems capable of inducing skeletal muscle hypertrophy via an enhanced program of gene expression (2), increased ribosomal-mediated translation (41), and satellite cell activation (8). Taken together, these results indicate that short recovery between bouts of high-intensity resistance exercise may suppress IGF-1 transcription and gene expression and attenuate anabolic processes.

Akt is a critical mediator of insulin/IGF signaling that, when activated, phosphorylates multiple substrates mediating important aspects of carbohydrate metabolism and protein synthesis and degradation (40). Here, we report a systematic downregulation in the phosphorylation state of Akt after repeated bouts of exercise, an effect that persisted for up to 48 h after multiple bouts undertaken in a single day (Fig. 2). A potential limitation of the present study was that we did not determine the isoform-specific activity of Akt. Nevertheless, given the proposed role for Akt in compensatory hypertrophy (5), we originally hypothesized that a cumulative increase in the phosphorylation of Aktser473 would occur after repeated bouts of resistance training. Moreover, Akt activity has previously been shown to both increase (12,17,33) or remain unchanged (12,18,33) in response to a variety of contractile stimuli. There are a number of possible explanations for the dephosphorylation of Akt in response to stacked resistance training bouts. The most likely one relates to the suppression and/or activation of kinases and phosphatases involved in the direct regulation of Akt activity.

Phosphorylation of the activation loops on threonine308 and the hydrophobic motif on serine473 is required for full activation of Akt (38). Insulin receptor substrate 1 (IRS-1) phosphorylation and insulin signaling have been shown to be impaired by increases in TNFα (16). Del Aguila and colleagues (16) have previously reported that IRS-1 signaling and Akt kinase activation is impaired by muscle damage–induced TNFα production. In the current study, we observed a sustained and consistent increase in TNFα after resistance training overload. TNFα seems to exert its effect on IRS-1 by activation of IKK in a p38 MAPK–dependent manner (15,21). In support of this contention, our findings show corresponding IKK and p38 phosphorylation concomitant with Akt dephosphorylation, indicating a possible TNFα-mediated effect on Akt activity. Collectively, these findings indicate that muscle damage after high-frequency resistance training and subsequent increases in TNFα pathway signaling may have exacerbated the persistent decrease in phosphorylation of Akt.

Akt is involved in the regulation of numerous cellular processes, including protein synthesis and degradation. Moreover, Akt has been shown to mediate protein synthesis and degradation via the Akt-mTOR and Akt-Foxo1 pathways, respectively, (34,39); yet, mTORser2448 and Foxo1ser256 phosphorylation were largely unaffected by the resistance training regimen or changes in Akt phosphorylation (Fig. 2). The mTOR–raptor complex seems to regulate cell growth through p70 S6K and 4E-BP1, whereas mTOR–rictor phosphorylates and activates Aktser473 (37). The downregulation of Akt phosphorylation and the increase in AMPK phosphorylation (Fig. 1) with the repeated training stimulus may have contributed to the lack of response in mTOR activity (42).

Phosphorylation of p70 S6K and 4E-BP1 has been shown to be regulated by Akt (5) and mTOR (6), and evidence suggests that these kinases may be fundamental for the control of cell size (35). Several p70 S6K substrates
exist, but, essentially, phosphorylated p70 S6K activates processes increasing translation efficiency while 4E-BP1 is deactivated with phosphorylation, inhibiting its role as a repressor of the translational machinery (5). Indeed, p70 S6K activation has been associated with an acute increase in protein synthesis and hypertrophy (17). Hence, a novel finding of the present study was the pronounced summation in p70 S6K phosphorylation with stacked resistance training that occurred independently of Akt or mTOR phosphorylation (Fig. 3). It is difficult to reconcile the increased p70 S6K phosphorylation in the absence of the activation of its upstream kinases. Indeed, previous studies have also shown p70 S6K phosphorylation in skeletal muscle independent of Akt/mTOR after resistance exercise and with muscular dysfunction (18,30). Accordingly, we examined the thr421/ser424 phosphorylation sites of p70 S6K because, under specific conditions, the Raf/MEK/ERK mitogen-activated protein kinase pathway has been implicated in mTOR-independent activation of p70 S6K via thr421/ser424 (24,32).

We observed a highly coordinated response of these distinct phosphorylation regions of p70 S6K after high-frequency resistance training (Fig. 3). Therefore, the possibility exists that the observed Akt/mTOR-independent phosphorylation of p70 S6K thr421/ser424-facilitated thr389 phosphorylation occurred as a mitogen- and stress-activated response to excessive overload (24). The observed changes in p70 S6K are in agreement with others showing the activation of p70 S6K in a resistance training–like stimulus in rodents and humans (3,5,17). However, further work is required to examine possible alternate pathways and physiological conditions that may mediate p70 S6K phosphorylation independently of Akt/mTOR phosphorylation after excessive training overload.

TNFα is an inflammatory cytokine shown to negatively affect anabolic processes by increasing the activity of protein-degradation pathways, destabilizing myogenic differentiation and altering transcriptional activity (22,27,28). Primary targets of TNFα signaling include the IKK and p38 MAPK (9,31). A fundamental role of IKK is the phosphorylation of the NF-kB inhibitor (IκB), which designates IκB for ubiquitination and subsequent degradation, releasing the transcription factor NF-κB to translocate to the nucleus and initiate the expression of a number of genes, including those involved in the regulation of skeletal muscle protein degradation such as MuRF1 (7). Similarly, TNFα-stimulated p38 phosphorylation also seems to induce gene expression of the E3 ubiquitin ligase MAFbx/Atrogin 1, promoting protein degradation in skeletal muscle (31).

Our in vivo data show that changes in IKK and p38 phosphorylation were highly coordinated with the responses of TNFα (Fig. 4). Specifically, the repeated stimulus during the stacked training protocol induced a significant increase in IKK and p38 phosphorylation after each discrete exercise bout. Accordingly, the sustained increase in the phosphorylation of kinases implicated in atrophy signaling likely increased the activity of protein degradation pathways, resulting in reduced net protein synthesis after the resistance training protocol. In addition, the initial and sustained increase in IKKK/TNFα pathway signaling with intermittent functional overload suggests that resistance exercise generates significant alterations in cellular homeostasis and a substantial proinflammatory response in skeletal muscle. Moreover, it is likely that the increases in IKK and TNFα in the present study were primarily the result of muscle damage per se, because a subgroup of animals subjected to an acute bout of prolonged swimming showed no alterations in IKK phosphorylation or IκBα degradation (V.G. Coffey and G.J. Lancaster, unpublished observations). Taken together, these results indicate that adequate recovery between resistance training bouts is important for reducing inflammatory signaling and subsequent protein degradation in skeletal muscle.

In conclusion, we have used a novel protocol to elucidate the discrete signaling and transcriptional events after exercise-induced contractile overload. We provide new information regarding the effects of resistance training frequency on signal transduction pathways and gene transcription governing hypertrophy/atrophy regulatory responses in skeletal muscle in vivo. We observed that the frequency of overload is an important factor determining subsequent adaptive events associated with muscle overload. Specifically, we provide experimental evidence to demonstrate that high-frequency loading suppressed IGF-1 transcription and Akt phosphorylation and exacerbated the expression of TNFα, IKKα/β activity, and p38 MAPK phosphorylation. The findings of the present study also indicate that an excessive accumulation of training stress has the capacity to impair specific anabolic processes for up to 48 h after exercise and generate an acute inflammatory response. Finally, our results may have important practical relevance in that the accumulation of repetitive overload/stress with inadequate recovery seems detrimental to the anabolic response while promoting inflammation, which may contribute to the etiology associated with overtraining in skeletal muscle.

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REFERENCES


36. PILANDER, N., R. DAMSKAARD, and H. PILSKAARD. Resistance


