Time course of the MAPK and PI3-kinase response within 24 h of skeletal muscle overload

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Carlson, Christian J., Zhiqiang Fan, Scott E. Gordon, and Frank W. Booth. Time course of the MAPK and PI3-kinase response within 24 h of skeletal muscle overload. J Appl Physiol 91: 2079–2087, 2001.—Knowledge of the molecular mechanisms by which skeletal muscle hypertrophies in response to increased mechanical loading may lead to the discovery of novel treatment strategies for muscle wasting and frailty. To gain insight into potential early signaling mechanisms associated with skeletal muscle hypertrophy, the temporal pattern of mitogen-activated protein kinase (MAPK) phosphorylation and phosphatidylinositol 3-kinase (PI3-kinase) activity during the first 24 h of muscle overload was determined in the rat slow-twitch soleus and fast-twitch plantaris muscles after ablation of the gastrocnemius muscle. p38α MAPK phosphorylation was elevated for the entire 24-h overload period in both muscles. In contrast, Erk 2 and p54 JNK phosphorylation were transiently increased by overload, returning to the levels of sham-operated controls by 24 h. PI3-kinase activity was increased by muscle overload only at 12 h of overload and only in the plantaris muscle. In summary, sustained elevation of p38α MAPK phosphorylation occurred early in response to muscle overload, identifying this pathway as a potential candidate for mediating early hypertrophic signals in response to skeletal muscle overload. hypertrophy; growth; signaling; mitogen-activated protein kinase; phosphatidylinositol 3-kinase

AGE- AND DISEASE-ASSOCIATED LOSSES in skeletal muscle mass decrease the quality of life and rob elderly humans of their independence, but resistance exercise can compensate for this loss (34). Skeletal muscle responds to muscle overload by increasing muscle fiber size, which is dependent on increased protein synthesis and proliferation of muscle satellite cells (26, 30). Thus discovery of the molecular mechanisms by which skeletal muscle senses and responds to changes in muscle loading may identify novel targets for medical treatment of elderly or recovering human patients. Recent advances have identified the calcineurin-signaling pathway as an important pathway for regulating muscle size (11); however, it has been demonstrated that activation of the calcineurin pathway by itself is not sufficient for muscle hypertrophy (12). Thus muscle hypertrophy may require the activation of additional signaling pathways. As mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3-kinase) pathways are capable of regulating cellular processes such as protein synthesis and cellular proliferation of numerous cell types (see Refs. 32 and 38 for reviews), the following experiments employed an animal model of skeletal muscle growth in young animals to gain a better understanding of the molecular mechanisms by which muscle can sense and respond to changes in the loads placed on it.

The MAPK family of kinases acts to transmit stimuli from the extracellular and cytoplasmic compartments to the nucleus (see Ref. 38 for review). The MAPK family can be divided into five subfamilies, including Erk 1/2, p38 MAPK, Jun NH2-terminal kinase (JNK), Erk 3/4, and Erk 5. Of these five subfamilies, the biochemistry and biological function of Erk 1/2, p38 MAPK, and JNK are better known and were chosen for examination in the present study. All MAPK pathways are comprised of a three-component module, i.e., consisting of three kinases that act in a sequential manner to transduce signals. The last component of the MAPK module is the MAPK itself. MAPKs require dual phosphorylation of a threonine-X-tyrosine motif (where X represents glutamate for Erk 1/2, glycine for p38 MAPK, and proline for JNK) within an activation-loop domain (9, 28, 29). Dual phosphorylation of the MAPK results in a conformational change that activates its kinase activity (8). Consequently, phosphorylated MAPKs can be translocated to the nucleus, where they are capable of regulating the activity of various transcription factors (9, 20, 29).

The effects of unloaded muscle contractions and aerobic exercise on members of the MAPK family have been examined. Muscle contractions and treadmill (aerobic) exercise can activate Erk 1/2, p38 MAPK, and JNK to varying degrees and time courses (3, 5, 6, 14, 31, 33, 39, 40). In addition, activation occurs in isolated...
muscles undergoing contractions, ruling out systemic factors as a significant contributor to contraction-stimulated MAPK activation (5, 31, 33, 39, 40). Recent experiments have identified potential roles for Erk 1/2, JNK, and p38 MAPK in facilitating cardiac muscle hypertrophy (7, 10, 17, 36, 42). Thus members of the MAPK family may also play a role in skeletal muscle hypertrophy in response to increased loading. To date, however, no experiments have examined activation of members of the MAPK family during overload-induced muscle hypertrophy in the whole animal.

It has recently been reported that resistance exercise can result in increased PI3-kinase activity at 6–24 but not at 1–3 h postexercise (19). One of the downstream targets of the PI3-kinase signaling pathway is regulation of protein synthesis rate (see Ref. 32 for review). It is well known that resistance exercise and muscle overload increase protein synthesis rate (26). Interestingly, acute elevations in unloaded contractile activity via electrical stimulation do not increase PI3-kinase activity in skeletal muscle (13). The failure of aerobic contractile activity to activate PI3-kinase is intriguing considering that aerobic or unloaded muscle contractions do not result in substantial muscle hypertrophy. Thus PI3-kinase signaling may be an important step in distinguishing the signaling events leading to distinctively different adaptations between aerobic and resistance exercise (i.e., muscle hypertrophy). Knowledge of the temporal activation pattern of the MAPK and PI3-kinase pathways may provide insight into the roles these pathways may play in regulating muscle hypertrophy in response to an overload stimulus. We hypothesized that potential candidates for transmitting an early hypertrophy stimulus would be identified on the basis of their temporal pattern of activation in response to muscle overload.

METHODS

Materials. Phosphorylation state-specific antibodies directed toward Erk 1/2, p38 MAPK, and JNK were purchased from Cell Signaling Technologies (formerly New England Biolabs, Beverly, MA). Antibodies directed to phosphorylation state-independent (total or pan) signaling molecules were purchased from Transduction Laboratories (pan-Erk; Lexington, KY) or Cell Signaling Technologies (p38α MAPK and JNK). Antibodies directed to the β5 regulatory subunit of PI3-kinase were purchased from Upstate Biotechnology (Piscataway, NJ). Protein A-sepharose, [γ-32P]ATP, and enhanced chemiluminescence reagents were purchased from Amersham-Pharmacia. Polyvinylidene difluoride transfer membranes were purchased from Millipore. All remaining chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO) or Fisher Scientific.

Animals and muscle overload. Fifty-six male Sprague-Dawley rats (~320 g body wt) were used for this experiment (Harlan, Indianapolis, IN). Animals were randomly divided into one of eight groups (n = 7/group), consisting of either sham-operated controls or overloaded groups with observation times of 1, 3, 12, and 24 h postrecovery from anesthesia. Animals in the overload groups were subjected to bilateral surgical ablation of the gastrocnemius muscle in the hindlimb (4). This procedure results in an overload stimulus on the remaining synergistic soleus and plantaris muscles which subsequently must compensate for the functional load of the ablated gastrocnemius muscle. To serve as a control, a separate set of animals underwent a sham surgery in which the gastrocnemius muscle was not removed. The overload stimulus typically results in significant increases in muscle wet weight and protein content of the soleus and plantaris compared with sham-operated controls as early as 24 h of overload (2, 4).

Surgical ablation of the gastrocnemius was performed under isoflurane anesthesia (3–5%) to facilitate a rapid recovery from the surgery and allow animals more immediate mobility. To ensure consistency between all animals in the time course experiments, the overload period (1, 3, 12, or 24 h) was initiated when the animal began to move freely around the cage, typically 20–30 min after surgery. To further ensure consistency between time points, as well as to ensure that the overload stimulus began at the start of the rats’ normal nocturnal activity pattern, all surgeries were performed in the late afternoon. During the overload period, animals in the 1-, 3-, and 12-h groups were caged individually in a dark room until the appropriate time of death. Animals in the 24-h overload group were subjected to their normal light-dark cycle starting with the dark cycle. Food and water were provided ad libitum to all animals. The University of Missouri Animal Care and Use Committee approved animal protocols.

Tissue collection and preparation. At the specified time (1–24 h postrecovery), the animals were anesthetized with ketamine, xylazine, and acepromazine (75, 3, and 5 mg/kg body wt, respectively), and the soleus, plantaris, and diaphragm muscles were removed from the animals. After tissue collection, the animals were euthanized by cervical dislocation while under anesthesia. Once removed from the animals, the muscle samples were frozen in liquid nitrogen and stored at −80°C until further preparation and analysis. Soleus and plantaris muscles from one leg, as well as a portion of the diaphragm, were later homogenized on ice with a Polytron mixer (Kinematica) in 0.1% Triton X-100, 50 mM HEPES, pH 7.4, 4 mM EGTA, 10 mM EDTA, 15 mM Na4P2O7, 100 mM β-glycerophosphate, 25 mM NaF, 5 mM Na3VO4, and 50 μg/ml leupeptin, pepstatin, and aprotinin. After homogenization, sample homogenates were aliquoted and stored at −80°C until further analysis. Muscle total protein content was calculated from the protein concentration of each sample and the final volume of the sample homogenate. Protein concentration of each sample was determined using an adaptation of the method of Lowry et al. (23) (Bio-Rad DC protein assay; Hercules, CA) with bovine serum albumin as a standard.

Analysis of phosphorylation status for MAPK family members. Total protein (50 μg) was loaded onto 10% SDS-PAGE gels. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Ann Arbor, MI) at 4°C. Phosphorylation status for Erk 2, p38 MAPK, and p54 JNK was determined by immunodetection with phospho-specific antibodies, each diluted 1:1,000 (vol/vol) in 5% bovine serum albumin-TBS-T (Tris-HCl, NaCl, Tween 20). The abundances of MAPK family members were determined with phosphorylation state-independent antibodies. Antibodies were diluted in 5% milk-TBS-T (1:5,000 for Erk 2 and 1:1,000 for p38α MAP and JNK). Bands were visualized by enhanced chemiluminescence (Amersham) and autoradiography.
Analysis of p85-associated PI3-kinase activity. PI3-kinase assay was performed as a modification of the protocol described by Upstate Biotechnology. Briefly, 1 mg of total muscle protein was diluted to 1 mg/ml in lysis buffer (137 mM NaCl, 20 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 0.1 mM NaVO₄, 50 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride) and gently agitated at 4°C for 30 min, followed by centrifugation at maximum speed in a refrigerated microcentrifuge for 10 min. The p85 regulatory subunit of PI3-kinase was immunoprecipitated from the resulting supernatant with a rabbit polyclonal antibody for 2 h. Immune complexes were collected by subsequent incubation with protein A-sepharose for 1 h at 4°C. After three washes with lysis buffer, immunoprecipitates were washed and resuspended in kinase buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA) in the presence of phosphatidylinositol (20 μg), MgCl₂ (10 mM), and [γ-32P]ATP for 10 min at 37°C. Reactions were stopped by the addition of CHCl₃-MeOH (vol/vol), and the organic phase was separated by centrifugation. The organic phase was spotted onto silicon thin-layer chromatography plates coated with potassium oxalate. The plates were developed by chromatography in CHCl₃-MeOH-H₂O-NH₄OH (60:47:11:3.2) (vol/vol). The dried plates were visualized by exposure to a phosphor screen (Storm Imager 860, Molecular Dynamics) and quantified by use of Imagequant software.

Densitometric analysis. Autoradiographs were scanned with a Molecular Dynamics personal scanning densitometer, and integrated optical densities of bands were determined with Imagequant software (Molecular Dynamics). Phosphorylation status of Erk 1/2, p38 MAPK, and p54 JNK was reported as integrated optical density (IOD) of phosphospecific autoradiograph divided by the IOD for the total protein autoradiograph.

Statistical analysis. Statistical analysis was performed with the use of ANOVA with repeated measures when appropriate. Post hoc analysis was performed by using Tukey’s post hoc analysis. Statistical significance was set at \( P < 0.05 \).

RESULTS

Muscle wet weights and total protein contents normalized by body weights. The soleus muscle did not increase in wet weight until 3 h of overload (left side of Fig. 1A), whereas the plantaris muscle wet weight was significantly increased at all time points (left side of Fig. 1B). Muscle total protein content was not different between sham and overloaded soleus muscles (right side of Fig. 1A); however, total protein content was increased by 24 h of overload in the plantaris (right side of Fig. 1B). These results are consistent with previous literature that has reported an increase in total muscle protein by 24 h of overload in the plantaris (2). The more rapid hypertrophy of the plantaris, compared with the soleus, is consistent with previous research indicating a more robust hypertrophy in the overloaded plantaris muscle (4).

MAPK phosphorylation. The protein abundances of Erk 2, p38 MAPK, and p54 JNK were not significantly different between sham-operated controls and

Fig. 1. Muscle overload results in increased muscle wet weight and protein content. Soleus (A) and plantaris (B) muscles were removed from animals after either a sham operation or muscle overload induced by bilateral surgical ablation of the gastrocnemius muscles at the indicated time point. A: soleus muscle wet weight (left) and total muscle protein content (right). B: plantaris muscle wet weight (left) and total muscle protein content (right). Data are means ± SE; \( n = 7 \) observations for each group. \*\( P < 0.05 \) compared with sham group at that time point.
overloaded muscles at any time point (representative data shown in Figs. 2–4).
p54 JNK phosphorylation was below detectable levels in sham-operated control plantaris and soleus muscles as well as the diaphragm muscles from either

**Fig. 2.** p54 JNK phosphorylation is transiently increased by muscle overload. Soleus (A) and plantaris (B) muscles were removed from animals after either a sham operation (S) or muscle overload (O) induced by bilateral surgical ablation of the gastrocnemius muscles at the indicated time point. Jun NH2-terminal kinase (JNK) phosphorylation was determined by Western blotting and immunodetection for JNK phosphorylated on Thr183 and Tyr185 (phospho-p54 JNK) during muscle overload. After phospho-specific immunodetection, membranes were stripped and reprobed with an antibody specific for JNK independent of phosphorylation state (pan-p54 JNK). JNK phosphorylation was detected in overloaded muscles (solid bars) and not in muscles from sham-operated controls. Phosphorylation status was calculated as phospho-specific integrated optical density (IOD) divided by pan IOD. Data are means ± SE; n = 7 observations for each group.

**Fig. 3.** Effects of muscle overload on phosphorylation of p38α MAPK. Soleus and plantaris muscles were removed from animals after either a sham operation (S) or muscle overload (O) induced by bilateral surgical ablation of the gastrocnemius muscles at the indicated time point. p38α MAPK phosphorylation was determined by Western blotting and immunodetection for p38 MAPK phosphorylated on Thr180 and Tyr182 (phospho-p38α MAPK) and for p38α MAPK independent of phosphorylation state (pan-p38α MAPK) during muscle overload. A: phosphorylation status of p38α MAPK during muscle overload of the soleus muscle. B: phosphorylation status of p38α MAPK during muscle overload of the plantaris muscle. Phosphorylation status was calculated as phospho-specific IOD divided by pan IOD. Data are means ± SE; n = 7 observations for each group. *P < 0.05 compared with sham group at that time point.
sham or overloaded animals. Numerous JNK isoforms exist, resulting from alternative splicing of three separate gene products, with apparent molecular masses of 46 and 54 kDa (see Ref. 38 for review). Muscle overload resulted in detectable phosphorylation of the 54-kDa isoform of JNK at the responding time points. Phosphorylation of the 46-kDa isoform was only detected in a subset of overloaded muscles (3 of 7 observations) at the 1-h observation. Thus protein and phosphorylation data are presented only for the 54-kDa JNK isoform (p54 JNK). p54 JNK phosphorylation peaked at 1 h of overload in both the soleus and plantaris muscles (Fig. 2, A and B). Thereafter, p54 JNK phosphorylation rapidly decreased and was undetectable by 12 h of overload for the plantaris muscle and by 24 h of overload for the soleus muscle.

Phosphorylation of p38 MAPK was not detected in the diaphragm muscle at any time point in either sham or overloaded animals. p38 MAPK phosphorylation was significantly increased by overload in the soleus and plantaris (Fig. 3). The p38 MAPK family consists of four isoforms (α, β, γ, and δ), each of which may have distinct cellular targets and physiological outcomes (27). Skeletal muscle is known to express at least the α-, β-, and γ-isoforms of p38 MAPK (22). The phospho-specific antibodies used in the present can detect dual-phosphorylation of the α, γ, and δ isoforms of p38 MAPK (16). Interestingly, a slower migrating band was often detected with the phospho-specific antibody in muscles from overloaded animals. Stripping and reprobing the blots with an antibody that recognizes the p38 MAPK isoform detected a single band that comigrates with the faster migrating phospho-p38 MAPK band. These results suggest that muscle overload is increasing the phosphorylation of p38 MAPK. It was not determined whether the slower migrating phospho-p38 MAPK band is another p38 MAPK isoform, possibly p38γ.

Despite an apparent effect of overload on phosphorylation of Erk 1 and 2, data are presented for Erk 2 only (Fig. 4), because an antibody suitable for quantifying the protein abundance of Erk 1 was not available. Erk 2 phosphorylation in the soleus muscle appeared to have a biphasic response to overload (Fig. 4B). Overload of the soleus muscle resulted in an increased phosphorylation of Erk 2 over sham-operated controls at 1, 3, and 12 h. At 3 h, Erk 2 phosphorylation in the overloaded soleus was significantly less than Erk 2 phosphorylation at 12 h of overload and had a tendency (P < 0.1) to be lower than Erk 2 phosphorylation at 1 h of overload. Erk 2 phosphorylation in the plantaris muscle was increased over that of sham-operated controls at 1 and 3 h (Fig. 4C). There was a nonsignificant trend (P = 0.07) for Erk 2 phosphorylation to be elevated at 12 h of overload. However, by 24 h, Erk 2
phosphorylation in the overloaded plantaris was not significantly different from that of sham-operated controls. Erk 2 phosphorylation was detected in the diaphragm muscles from both sham-operated and overloaded animals and was not different between overloaded animals and sham-operated controls at any time point (Fig. 4D). Erk 2 phosphorylation in the diaphragms from sham-operated animals was increased at 3- (P < 0.05) and 12-h (P = 0.052) time points compared with the 1-h time point.

p85-Associated PI3-kinase activity. p85-Associated PI3-kinase activity exhibited a complex response to muscle overload (Fig. 5B). p85-Associated PI3-kinase activity was significantly increased in the overloaded plantaris muscles at the 12-h time point, but no differences were found for the soleus and diaphragm muscles from overloaded animals compared with sham-operated controls at 12 h. Increases in p85-associated PI3-kinase activity at 12 h in the plantaris muscle were not due to an increased protein abundance of p85 (Fig. 5C). At 24 h of overload, there was a nonsignificant tendency (P = 0.07) for p85-associated PI3-kinase activity to be elevated in the plantaris. p85-Associated PI3-kinase activity was not different from sham-operated controls in the soleus or diaphragm muscles at 24 h of overload. This delayed increase is similar to the effects of resistance exercise, which does not increase PI3-kinase activity until 6 h postexercise (19). At 1 h of overload, p85-associated PI3-kinase activity was significantly decreased in the diaphragms from overloaded animals compared with diaphragms from sham-operated controls. At 3 h of overload, p85-associated PI3-kinase activities were decreased in all muscles (soleus, plantaris, and diaphragm) compared with muscles from sham-operated animals. The p85 PI3-kinase antibodies used in the present experiment were directed against the full-length protein and are capable of immunoprecipitating and detecting other isoforms of the regulatory subunit of PI3-kinase such as p55 and p50 PI3-kinase. The present experiment focused on the p85 subunit (Fig. 5C), and, although the expression level of p85 was not altered by overload, it cannot be ruled out that an alteration in the expression level of p55 and p50 isoforms contributed to the increased activity observed at 12 h of overload.

DISCUSSION

The experiments report here for the first time that skeletal muscle overload in living animals increases the phosphorylation of p54 JNK, p38α MAPK, and Erk 2 proteins. However, differences in the temporal pattern of phosphorylation were observed for p54 JNK, p38α MAPK, and Erk 2, suggesting that muscle overload may involve a complex regulation of these MAPKs. The phosphorylation status of p54 JNK and Erk 2 were transiently increased from 1 to 12 h of muscle overload. In contrast, p38α MAPK phosphorylation was elevated at all durations (1–24 h) of muscle overload examined. This suggests that p38α MAPK may be also involved with regulating gene expression beyond the initial (i.e., 1–12 h) signaling events associated with muscle overload. This may be significant because muscle overload induced by surgical ablation of synergistic muscles results in a long-lasting stimulus that promotes muscle growth for many weeks (2, 4, 26). Thus the consistent overload-induced phosphorylation of p38α MAPK, compared with Erk 2 or p54 JNK that were more transient in nature, suggests that p38α MAPK may have a unique role in muscle growth.
The observation that muscle contraction in vitro can activate the MAPK family indicates that stimuli within the muscle are sufficient to activate these pathways without the influence of systemic factors (5, 31, 33, 39, 40). Likewise, in the present experiment, overload resulted in phosphorylation of p54 JNK and p38α MAPK, yet phosphorylation of these proteins was not detected in the diaphragm muscle, indicating that these effects of overload are specific to the overload response and not to a systemic factor. In contrast to p54 JNK and p38α MAPK, Erk 2 phosphorylation was detected in the diaphragm muscles from sham-operated and overloaded animals. Erk 2 phosphorylation was transiently increased in the diaphragm muscles from sham-operated controls over the course of the experiment. However, there were no significant differences in Erk 2 phosphorylation between diaphragm muscles from sham-operated animals and overloaded animals. Because there were no differences in Erk 2 phosphorylation in the diaphragms between sham-operated or overloaded animals, it is unlikely that any systemic factor was a major contributor to the overload-induced increase in Erk 2 phosphorylation observed in the soleus and plantaris muscles.

Recently it has been reported that, after a marathon, the phosphorylation status of the p53γ MAPK isoform is preferentially increased compared with p38α (6). In the present experiment, muscle overload appeared to result consistently in increased phosphorylation of the p38α MAPK isoform, with occasional increases in a slower migrating band, which may be p38γ MAPK. It is possible that these differences in isoform-specific phosphorylation of different p38 MAPK isoforms may be dependent on the type of exercise stimulus (i.e., endurance exercise vs. muscle hypertrophy) or may reflect differences between species (i.e., human vs. rat).

Previously reported in vitro muscle differentiation experiments support a potential role for p38 MAPK during skeletal muscle hypertrophy. During in vitro muscle differentiation, p38 MAPK is rapidly activated, and this activation is maintained during the entire process of myotube formation (21, 41). p38 MAPK targets some muscle-specific transcription factors such as myocyte enhancer factor 2C (MEF2C) and MyoD (17, 27). Skeletal muscle hypertrophy is associated with increased expression of MyoD and MEF2 (1, 12); thus the activation of p38α MAPK during muscle overload may contribute to the regulation of gene expression via myogenic transcription factors.

Recently, the calcineurin signaling pathway has been shown to play an important role in cardiac and skeletal muscle hypertrophy (11, 24). Reductions in calcineurin activity prevented the increase in muscle mass and fiber cross-sectional area in response to muscle overload (11), suggesting that calcineurin activity is necessary for adult skeletal muscle hypertrophy. However, Dunn et al. (12) recently demonstrated that constitutive activation of calcineurin does not result in increased muscle mass, nor does it potentiate the overload stimulus. Furthermore, Naya et al. (25) found that other signals are necessary, in addition to calcineurin, to induce muscle hypertrophy. Taken together, these data indicate that calcineurin activation is necessary, but not sufficient, for maximal skeletal muscle hypertrophy, and that additional signaling pathways may be necessary, as suggested by Naya et al. The present experiment identifies p38α MAPK as a potential parallel pathway with calcineurin because it is activated by skeletal muscle overload. Furthermore, the transcription factor MEF2 is a common substrate for both calcineurin and p38 MAPK, indicating the potential for calcineurin and p38 MAPK signals to integrate at this transcription factor during muscle hypertrophy.

Several investigators have observed muscle damage as well as cellular infiltration of overloaded skeletal muscle (2). Many stimuli are capable of activating the MAPKs. Notably, JNK and p38 MAPK, also described as stress-activated protein kinases, are known to be activated by numerous inflammatory cytokines as well as cytotoxic stimuli such as osmotic shock and oxidative damage (27, 38). In the present experiment, overload resulted in phosphorylation of p54 JNK and p38α MAPK, yet phosphorylation of these proteins was not detected in the diaphragm muscle, indicating that these effects of overload are specific to the overload response and are not due to a systemic factor. Although the presence of a local inflammatory response during hypertrophy complicates the interpretation, experimental evidence supports the speculation that the inflammatory response is a necessary component of muscle regeneration and hypertrophy. It has been proposed that repeated exposures to muscle damage, and subsequent regeneration, are a necessary component for muscle growth (37). Furthermore, macrophages, which are known to infiltrate overloaded skeletal muscle, are capable of releasing factors that can activate muscle satellite cells in vitro (15).

PI3-kinase activity exhibited a complex response to the overload stimulus. At 3 h of overload, PI3-kinase activity was decreased in the overloaded soleus and plantaris muscles. This decrease was also observed in the diaphragm muscles from overloaded animals compared with the diaphragms from sham-operated animals. This “global” downregulation of PI3-kinase activity could have been caused by systemic factors. The amount of trauma caused by removing the gastrocnemius muscle from the overloaded animals is most likely greater than that seen in sham-operated controls and thus may contribute to a greater stress response in the overloaded animals. It is possible that some systemic factor, such as a stress hormone or inflammatory cytokine, may be involved with a global and transient inhibition of PI3-kinase activity, as previously described occurring with MAPK.

Overload resulted in a significant increase in PI3-kinase activity in the overloaded plantaris muscle at 12 h of overload whereas PI3-kinase activity was not different in the diaphragm and soleus muscles between sham-operated controls and overloaded animals at this time point. Although it is not known how ablation of the gastrocnemius muscle affects the recruitment patterns of the soleus and plantaris, it is well known that

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a slow-twitch muscle is recruited more often than a fast-twitch muscle in an unmanipulated animal (18). It is therefore possible that the removal of the gastrocnemius muscle increases the absolute neural activation of the plantaris muscle (a fast-twitch muscle) to a greater extent than the soleus muscle (a slow-twitch muscle). This speculation is also supported by the more rapid increase in plantaris wet weight and protein content compared with the soleus muscle.

The data in the present report suggest that multiple signaling pathways may play a significant role in overload-induced skeletal muscle hypertrophy. It is obvious that the activation of these pathways during a hypertrophy stimulus is a complex process not clearly understood at this time.

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