Effects of exercise on mitogen- and stress-activated kinase signal transduction in human skeletal muscle

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1Department of Clinical Physiology, Karolinska Hospital; 2Department of Physiology and Pharmacology, Karolinska Institutet; 3Department of Sport and Health Sciences, Stockholm University College of Physical Education and Sports, Stockholm, Sweden; and 4Department of Biochemistry, University of Dundee, United Kingdom

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Krook, Anna, Ulrika Widengren, Xin Jian Jiang, Jan Henriksson, Harriet Wallberg-Henriksson, Dario Alessi, and Juleen R. Zierath. Effects of exercise on mitogen- and stress-activated kinase signal transduction in human skeletal muscle. Am J Physiol Regulatory Integrative Comp Physiol 279: R1716–R1721, 2000.—Exercise/contraction is a powerful stimulator of mitogen-activated protein (MAP) kinase cascades in skeletal muscle. Little is known regarding the physiological activation of enzymes downstream of MAP kinase. We investigated whether acute exercise results in activation of mitogen- and stress-activated kinases (MSK) 1 and 2, p90 ribosomal S6 kinase (p90rsk), and MAP kinase-activated protein kinase 2 (MAPKAPK2). Muscle biopsies were obtained from healthy volunteers before, during, and after 60 min one-leg cycle ergometry, from exercising and resting legs. MSK1 and MSK2 activities were increased 400–500% and 200–300%, respectively, in exercised muscle (P<0.05 vs. rest). A dramatic increase in activity of p90rsk (MAPKAPK1) (>2,500%), and to a lesser extent MAPKAP2 (300%), was noted with exercise (P<0.05 vs. rest). MSK1, MSK2, p90rsk, and MAPKAP2 activities were sustained throughout exercise. Exercise-induced activation of these enzymes was limited to working muscle, indicating that local rather than systemic factors activate these signaling cascades. Thus physical exercise leads to activation of multiple enzymes downstream of MAP kinase.

Exercise training increases muscle mass (5, 6), improves glucose tolerance (19, 22), and increases glucose uptake (18, 22, 27). These exercise-induced adaptations may be associated with changes in expression of key proteins involved in metabolism (9, 18–21). Recent interest has focused on delineating exercise-responsive signaling pathways in skeletal muscle (2, 3, 14, 28). The mitogen-activated protein (MAP) kinase signaling network regulates gene transcription and protein synthesis (8, 10, 11, 17), and this may be a possible mechanism by which exercise/muscle contraction leads to increased expression of muscle proteins (2, 3, 14, 28).

The classic MAP kinase enzymes, p42 and p44 extracellular-regulated kinase (ERK) MAP kinase, are activated by polypeptide growth factors and tumor-promoting phorbol esters (11, 17). Other members of the MAP kinase family include the stress-activated protein kinases (SAPK), SAPK1/JNK, and SAPK2/p38, which integrate intracellular signals from diverse extracellular stimuli and/or various forms of cellular stress (15, 24). Signaling cascades leading to the MAP kinase pathways are activated in response to muscle contraction. p42/44 ERK and p38 MAP kinase are activated in human and rat skeletal muscle by exercise (2, 3, 14, 28). Furthermore, muscle contraction evoked by electrical stimulation leads to the activation of p42/44 ERK and p38 MAP kinase (23). Nevertheless, the downstream regulation of MAP kinase signal cascades in response to exercise is not fully known.

The aim of this study was to determine whether downstream targets of MAP kinase enzymes are activated in response to physical exercise in human skeletal muscle. We determined whether two newly identified enzymes, mitogen- and stress-activated protein kinases (MSK) 1 and 2, are activated in response to muscle contraction. These enzymes are directly activated both in vitro and in vivo by p42/44 ERK and p38 MAP kinase in cultured cells (13). However, in vivo activation of either MSK1 or MSK2 has not been demonstrated in skeletal muscle in response to physiological stress such as that evoked by exercise. In addition, we assessed whether two other known targets of MAP kinase, p90 ribosomal S6 kinase (p90rsk, also known as MAPKAPK1) (30) and MAP kinase-activated protein kinase 2 (MAPKAPK2) (4, 12), are activated in human skeletal muscle in response to exercise. These enzymes are downstream targets of p42/44 ERK and p38 MAP kinase, respectively.

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METHODS

**Subjects.** Fifteen healthy young volunteers (6 females and 9 males) participated in the study [age, 25 ± 1 years; weight, 71.5 ± 2.7 kg; height, 176 ± 2 cm; body mass index (BMI) 23.1 ± 0.6 kg/m²]. None of the subjects was engaged in any regular exercise training programs. Subjects were instructed not to engage in exercise for 48 h before being studied. The Ethical Committee at Karolinska Institutet and Karolinska Hospital approved the study protocol, and informed consent was obtained from all subjects prior to participation.

**Experimental protocol.** Maximal oxygen uptake (VO₂\text{max}) for one-leg and two-leg cycle ergometry was determined 3 days before the experimental protocol was undertaken. VO₂\text{max} was 2.8 ± 0.4 and 3.8 ± 0.5 l/min for one- and two-leg exercise, respectively. Subjects performed one-leg cycle ergometry at a load corresponding to ~70% of one leg VO₂\text{max}. After local anesthesia, an incision (5 mm long, 10 mm deep) was made in the skin and muscle fascia, and a muscle biopsy (20–100 mg) was obtained from the vastus lateralis portion of the quadriceps femoris by means of a Weil-Blakesley conchotome. Each biopsy was removed from a separate incision site, 3 cm apart. Muscle specimens were obtained (Fig. 1) from the left vastus lateralis muscle of the previously exercised leg at 75 min (solid squares) or 9 min after (n = 12) or 3 min after (n = 8) exercise. No more than three biopsies were obtained from the exercised leg from any one subject. Muscle tissue was immediately frozen in liquid nitrogen and stored at −80°C.

**Analysis.** Portions of skeletal muscle biopsies (20–30 mg) were homogenized in ice-cold buffer A (50 mM Tris-HCl, pH 7.5, 0.1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 10 mM β-glycerophosphate, 5 mM sodium pyrophosphate, 0.1% 2-mercaptoethanol, and 1 μM microcystin). Homogenates were rotated for 60 min at 4°C and centrifuged at 13,000 g for 10 min at 4°C. The supernatant was removed, and protein concentration was determined (Protein Kit; Bio-Rad, Richmond, CA). From the remaining homogenate described above, aliquots of the supernatant (500 μg for MSK1 and MAPKAPK2, 700 μg for MSK2, and 200 μg for p90rsk) were immunoprecipitated for 30 min at 4°C with the appropriate antibody previously equilibrated with protein G-Sepharose in buffer A. Immunoprecipitates were washed three times in buffer A containing 0.5 M NaCl, two times in buffer B (50 mM Tris-HCl, pH 7.5, 0.03% Brij-35, 0.1 mM EGTA, 0.1% 2-mercaptoethanol) and resuspended in 30 μl of kinase buffer [50 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 17 μM PKA inhibitor peptide (peptide inhibitor of cAMP-dependent protein kinase; Sigma, St. Louis, MO), 16.7 mM Mg(C₂H₃O₂)₂·4H₂O, supplemented with 50 μM peptide substrate (Crosstide for MSK1, MSK2, and p90rsk; KKLNRTLSVA peptide for MAPKAPK2), and 2 μCi [γ-³²P]ATP]. After 10-min incubation at 30°C, the reaction was terminated, and ³²P incorporation into the peptide substrate was determined by resolving the reaction products on a 40% acrylamide gel. The gel was visualized on a PhosphorImager (Bio-Rad), and the band corresponding to the peptide substrate was quantitated. Results were related to percent of value obtained in a standard sample (rat soleus muscle exposed to 600 mM mannitol for 20 min; hyperosmotic stress).

**Materials.** Antibodies were prepared as described previously for p90rsk (1), MSK1 and MSK2 (13), and MAPKAPK2 (1). All other reagents were analytical grade (Sigma).

**Statistical analysis.** Activation of enzymes was analyzed by two-way ANOVA. Results were considered significant at P < 0.05. When a significant F ratio was found, Newman-Keuls post hoc test was used to compare mean values.

RESULTS

**Subject characteristics and exercise performance.** A detailed description of the physical fitness level and blood chemistry profile of the study participants before and after exercise has been previously reported (28).

**MSK1 and MSK2 activity.** Exercise leads to phosphorylation and activation of p42/44 ERK and p38 MAP kinase in human skeletal muscle (3, 28). Here we determine the effects of exercise on kinases thought to function downstream of p42/44 ERK and p38 MAP kinase. MSK1 and MSK2 activity was assessed in skeletal muscle biopsies using an immunocomplex assay. Acute exercise led to a profound (400–500%) increase in MSK1 activity in working muscle (P < 0.01, Fig. 2), with peak activity observed at 60 min of exercise (P < 0.01 vs. rest). Acute exercise also resulted in

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**Fig. 1.** Schematic representation of the protocol used to obtain skeletal muscle following one-legged bicycle exercise. Arrows represent biopsy sampling. No more than 3 biopsies were obtained from the right vastus lateralis muscle (exercised leg) from each subject.

**Fig. 2.** Exercise-induced activation of mitogen- and stress-activated kinase 1 (MSK1) in human skeletal muscle. Biopsies from the left vastus lateralis muscle (rested leg; open squares) were obtained from the study participants before (n = 12) or 3 min after (n = 8) exercise. Biopsies from working right vastus lateralis muscle (exercised leg; solid squares) were obtained after 10 (n = 3), 30 (n = 10), or 60 (n = 9) min of exercise. Exercise was terminated after 60 min. Subjects rested for 15 min (recovery), and a biopsy was obtained from the previously exercised leg at 75 min (n = 3). MSK1 activity was assessed against a peptide substrate. **Inset:** representative PhosphorImager (acquired on Bio-Rad Molecular Analyst version 1.5) of the band corresponding to phosphorylated peptide substrate. Values are means ± SE. Exercise effect was significant as assessed by 2-way ANOVA (P < 0.01).
Acute exercise led to an increase in MAPKAPK2 activity in working muscle (Fig. 3). Biopsies were obtained from the left vastus lateralis muscle (rested leg; open squares) before \((n = 11)\) or 3 min after \((n = 9)\) exercise. Biopsies from working right vastus lateralis muscle (exercised leg; solid square) were obtained after 10 \((n = 3)\), 30 \((n = 10)\), or 60 \((n = 7)\) min of exercise. Exercise was terminated after 60 min. MSK2 activity was assessed against a peptide substrate. Inset: a representative PhosphorImage of the band corresponding to phosphorylated peptide substrate. Values are means ± SE. Exercise effect was significant as assessed by 2-way ANOVA \((P < 0.05)\). MSK2 activity could not be assessed following cessation of exercise due to insufficient sample.

A marked 200–300% increase in MSK2 activity \((P < 0.05, \text{Fig. 3})\). MSK2 activity increased throughout exercise, with peak activity observed at 60 min \((P < 0.05 \text{ vs. rest})\). We utilized a one-legged bicycle protocol to determine whether exercise-induced phosphorylation of MSK1 and MSK2 was due to local or systemic factors. MSK1 and MSK2 activity was only increased in exercised muscle, providing evidence that local rather than systemic factors mediate these effects.

**p90rsk and MAPKAPK2 activity.** Acute exercise led to a dramatic (>2,500%) increase in p90rsk activity in the exercising leg \((P < 0.01, \text{Fig. 4})\), with a maximal increase observed at 60 min \((P < 0.05 \text{ vs. rest})\). p90rsk activity remained elevated in working muscle 15 min postexercise. The pattern and magnitude of activation of p90rsk parallel our previous report of exercise-induced phosphorylation of p42/44 ERK (28). This observation is consistent with studies of cultured cells showing that activation of p90rsk is primarily under the control of the ERK pathway (16, 25). Acute exercise also led to an increase in MAPKAPK2 activity in working muscle \((P < 0.05, \text{Fig. 5})\). MAPKAPK2 activity reached a maximal 330% increase over nonexercised levels at 60 min of exercise \((P < 0.05 \text{ vs. rest})\) and tended to decrease with cessation of exercise. As for MSK1, MSK2, and p90rsk, exercise-induced activation of MAPKAPK2 was only observed in working muscle, supporting the concept that local rather than systemic factors mediate exercise-induced MAP kinase signal transduction.

**DISCUSSION**

Activation of the different MAP kinase pathways by physical exercise could be important in the regulation of transcriptional events in skeletal muscle. To date, there is limited information regarding the regulation and activation of MAP kinase signaling cascades in human muscle. Muscle contraction and physical exercise are powerful stimulators of several parallel MAP kinase enzymes (2, 3, 14, 23, 28). However, the downstream molecular targets have not been identified in human skeletal muscle. Here...
we provide evidence of exercise-induced signal transduction through MSK1, MSK2, p90rsk, and MAPKAPK2, known substrates of p42/44 ERK and p38 MAP kinase (12, 13, 16, 23, 29, 30). Furthermore, through use of a one-leg cycling protocol, we show that activation of these signaling cascades is due to local rather than systemic effects.

We have previously demonstrated that both p42/44 ERK and p38 MAP kinase are phosphorylated in human skeletal muscle in response to exercise (28). However, the pattern of exercise-induced phosphorylation of these two kinases was different (28). Phosphorylation of p42/44 ERK MAP kinase was rapid, restricted to the working muscle, and returned to basal levels after cessation of exercise. In contrast, p38 MAP kinase phosphorylation was induced more slowly, and this was not restricted to the exercising muscle, suggesting that systemic factors could play a role in activation of p38 MAP kinase. Here we show that MSK1 and MSK2 were rapidly activated in response to exercise, and activation was limited to exercising muscle, similar to p42/44 ERK MAP kinase. This suggests that in human skeletal muscle, exercise-mediated activation of MSK1 and MSK2 may occur primarily via a p42/44 ERK MAP kinase-dependent pathway, rather than a p38 MAP kinase-dependent pathway. Furthermore, local, rather than systemic, factors elicit MSK1 and MSK2 activation with exercise. Peak phosphorylation of p42/44 ERK MAP kinase was noted at 30 min exercise (28), whereas peak MSK1 and MSK2 activity was noted at 60 min. This time course is consistent with the observations that MSK1 and MSK2 are downstream targets of p42/44 ERK MAP kinase (13, 23). The physiological role of activated MSK1 and MSK2 in human muscle is unknown. Activated MSK1 has been shown to phosphorylate histone H3/HMG-14 (26) as well as the transcription factor CAMP-responsive element binding protein (CREB) (13), indicating that it may play a role in regulation of gene expression. However, we did not observe increased CREB phosphorylation in response to exercise (28). Thus, at least in skeletal muscle, activation of MSK1 may not be sufficient to mediate phosphorylation of CREB. Alternatively, MSK1 may have different functions in different tissues.

To date, little is known of the regulation of the downstream molecular targets of p42/44 ERK and p38 MAP kinase in skeletal muscle. Specific inhibitors of ERK and p38 MAP kinase (PD-98059 and SB-203580, respectively) are useful reagents to determine the downstream components of the MAP kinase signaling cascade in response to exercise/contraction (1, 12, 23). In isolated rat epitrochlearis muscle, contraction, evoked by electrical stimulation, leads to phosphorylation of p42/44 ERK MAP kinase and a parallel activation of the downstream target p90rsk (23). Inhibition of ERK by PD-98059 completely abolished the effect of contraction on p90rsk activity (23). Thus muscle contraction-induced p90rsk activity is ERK dependent. In human skeletal muscle, the exercise-induced activation of p90rsk (present study) closely follows the phosphorylation of p42/44 ERK MAP kinase (28). This finding is consistent with the notion that p90rsk is a downstream target of p42/44 ERK MAP kinase.

MAPKAPK2 is a downstream target of p38 MAP kinase (12, 16, 23). Inhibition of the p38 MAP kinase pathway by SB-203580 ablates electrically stimulated muscle-contraction-induced MAPKAPK2 activity (23). Clearly, inhibitor studies are not possible to perform in exercising humans. However, our finding that exercise increases MAPKAPK2 activity in skeletal muscle is consistent with previous reports in electrically stimulated muscle (23). Furthermore, we provide the first evidence that this molecular target of p38 MAP kinase can be activated in vivo, in response to a physiologically relevant stimulus.

Skeletal muscle is highly adaptable to the demands of exercise training. Changes in mRNA and protein expression of key components of glucose metabolism occur in response to both acute and chronic exercise. Acute exercise is associated with increased hexokinase II, GLUT4, and glycogenin gene expression (mRNA) in skeletal muscle (20). Regular aerobic exercise training is associated with increased GLUT4 protein expression and improved glucose tolerance in glucose-intolerant people (19). These changes are noted in response to an exercise bout that mimics the intensity and duration typical of the average nonathlete, similar to that prescribed in the present study. Further studies are warranted to directly link MAP kinase signaling with these exercise-induced changes in mRNA and protein expression.

Exercise is a potent activator of the MAP kinase cascade, presumably noted in response to the physiological stress induced by contraction in skeletal muscle (28). This potent induction is comparable to activation measured in cells stimulated with supraphysiological ligand concentrations or where key signaling elements have been experimentally overexpressed (7, 11). Thus MAP kinase signaling pathways are likely to be mediators of exercise-induced changes in gene expression in skeletal muscle; however, this remains to be directly established. Our finding that exercise increases MSK1, MSK2, p90rsk, and MAPKAPK2 activity in skeletal muscle offers support for the hypothesis that contraction-induced MAP kinase signaling increases transcriptional activity, as these kinases have been implicated in the phosphorylation of transcription factors (11, 13).

In conclusion, exercise is a physiological stimulus that leads to a profound, in vivo activation of MSK1, MSK2, p90rsk, and MAPKAPK2 in human skeletal muscle. This coordinated activation of MAP kinase signaling cascades may mediate exercise-induced changes in gene expression and alterations in muscle metabolism.

**Perspectives**

Moderate exercise leads to a variety of acute and chronic changes in the muscle phenotype. MAP kinase
signaling pathways provide a potential signaling mechanism to account for exercise-induced regulation of genes in skeletal muscle. Muscle contraction is an extremely powerful activator of both p38 and ERK MAP kinase and several downstream enzymes. Specific inhibitors of ERK and p38 may be useful to determine whether MAP kinase signaling systems are directly involved in transcriptional regulation of important genes in skeletal muscle in response to exercise. In addition, future studies should be directed toward identifying the proteins involved in exercise-responsive MAP kinase signaling networks. Since exercise-induced activation of MAP kinase is limited to working muscle, local rather than systemic factors appear to mediate this signaling cascade. However, the direct link between muscle contraction and activation of MAP kinase signaling is not fully defined. This mechanism will be necessary to unravel, as it can be important for our full understanding of the exercise effects on gene regulation. The link between muscle contraction and MAP kinase activation may offer a potential site of pharmacological intervention to control expression of proteins that may prevent muscle wasting or improve glucose homeostasis.

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