Prior exercise increases basal and insulin-induced p38 mitogen-activated protein kinase phosphorylation in human skeletal muscle

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Thong, Farah S. L., Wim Derave, Birgitte Ursø, Bente Kiens, and Erik A. Richter. Prior exercise increases basal and insulin-induced p38 mitogen-activated protein kinase phosphorylation in human skeletal muscle. J Appl Physiol 94: 2337–2341, 2003.—We have examined the effects of insulin on p38 mitogen-activated protein kinase (MAPK) phosphorylation in human skeletal muscle and the effects of prior exercise hereon. Seven men performed 1-h one-legged knee extensor exercise 3 h before the initiation of a 100-min euglycemic-hyperinsulinemic (600 pmol/l) clamp. Glucose uptake across the legs was measured with the leg balance technique, and muscle biopsies were obtained from the rested and exercised vastus lateralis before and during insulin infusion. Net glucose uptake during the clamp was 50% higher (P < 0.05) in p38 MAPK phosphorylation in the exercised leg compared with the rested leg before and during insulin infusion. We conclude that a physiological concentration of insulin causes modest but sustained activation of the p38 MAPK pathway in human skeletal muscle.

The p38 mitogen-activated protein kinase (MAPK) cascade represents one of the major signal transduction pathways used by eukaryotic cells to transduce extracellular signals into cellular responses. It belongs to a superfamily consisting of at least three parallel and distinct MAPK pathways, which include the extracellular regulated kinases and the two stress-activated protein kinase cascades c-Jun NH2-terminal kinase and p38 (reviewed in Ref. 25). The p38 subgroup is implicated in the regulation of numerous cellular processes (4, 10, 16, 30). Recently, a potential role of p38 MAPK in the contraction and insulin stimulation of glucose transport in skeletal muscle has been suggested (2, 22, 23, 28). Insulin and contractions were found to stimulate the kinase activity and phosphorylation status of p38 MAPK in rat skeletal muscle, and application of an inhibitor of p38 MAPK (SB-203580) inhibited glucose transport in response to both separate stimuli (22). Several lines of evidence suggest that p38 MAPK is located at a convergence point of exercise and insulin signals to glucose transport stimulation and that it is involved in the signal leading to increased intrinsic activity (i.e., catalytic activity) of the glucose transporter protein (GLUT-4) at the surface membrane rather than in the signal leading to translocation of GLUT-4 to the surface membrane (21, 23). However, the stimulatory effect of insulin on p38 MAPK and its relation to glucose transport are still debated (6, 7, 15). Moreover, in vivo data showing the ability of a physiological insulin concentration to stimulate p38 MAPK in human skeletal muscle are still lacking.

It is well established that a single bout of exercise increases the insulin sensitivity of muscle glucose uptake in the postexercise period (19, 20). However, the molecular mechanism for this phenomenon still remains to be elucidated (18). Wojtaszewski et al. (26, 27) have shown that increased activation of the proximal cascade of insulin signaling intermediates, which have been related to glucose transport stimulation, cannot explain the postexercise sensitization to insulin in muscle. Interestingly, p38 MAPK seems to provide a likely target for exploring the increased insulin sensitivity after exercise since the enzyme supposedly lies at a convergence point of both stimuli (22).

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The present study aimed to investigate whether a high physiological dose of insulin can increase p38 MAPK phosphorylation in human skeletal muscle. Furthermore, it was hypothesized that a single bout of exercise could enhance p38 MAPK phosphorylation during a euglycemic hyperinsulinemic clamp initiated 3 h after exercise.

MATERIALS AND METHODS

Subjects. Seven healthy, moderately active men were recruited to participate in this study. The subjects' age, weight, height, and body mass index were 26 ± 2 yr, 78 ± 4 kg, 180 ± 3 cm, and 24 ± 1 kg/m², respectively. This study was approved by the Ethics Committee of Copenhagen and Frederiksborg County, and informed consent was obtained from each subject. Before determination of peak work capacity via an incremental knee extensor test, the subjects were first accustomed to the one-legged knee extensor apparatus on three different occasions. On the day of the experiment, the subjects ate a light breakfast 2 h before arriving at the laboratory at 7 AM. The results presented in this study are part of a larger study dealing with the effects of caffeine on insulin-stimulated glucose uptake and insulin signaling, which has partly been published previously (24).

Experimental protocol. The subjects performed 60 min of repeated (1 kick/s) one-legged knee extensor exercise alternating every 5 min at workloads eliciting 75 and 100% of the knee extensor peak work capacity. The selection of the exercising leg was randomized. Three subjects exercised the left leg, whereas four subjects exercised the right leg. Subjects rested in a supine position after the exercise, and Teflon catheters were inserted below the inguinal ligament in the femoral artery and bilateral femoral veins. A thermistor (Baxter) was inserted through each femoral venous catheter into each femoral arterial catheter, and arterial blood flow was measured in both thighs by using a modified thermodilution method, as previously described (20). Pneumatic cuffs were placed below the knees and were inflated to 230 mmHg during blood sampling and blood flow measurements. Biopsies from the rested (nonexercised) and exercised legs were taken under local anesthesia from the vastus lateralis at 0, 30, 50, and 100 min after the initiation of the insulin clamp. Blood flow was measured in both thighs by using a modified thermodilution method, as previously described (20). Pneumatic cuffs were placed below the knees and were inflated to 230 mmHg during blood sampling and blood flow measurements. Biopsies from the rested (nonexercised) and exercised legs were taken under local anesthesia from the vastus lateralis at 0, 30, and 100 min of insulin infusion and were quickly frozen in liquid nitrogen. The muscle biopsies from the rested and exercised legs were obtained within 30 s at each time point.

Analytical procedures. Plasma and blood glucose were determined in duplicates by using a dual-channel glucose analyzer (YSI-2700Select, Yellow Springs Instruments, Yellow Springs, OH). Muscle was freeze-dried and subsequently dissected under a stereomicroscope by removing any blood, connective tissue, or fat, leaving the muscle fibers for analysis. Muscle glycogen content was determined by a standard enzymatic fluorometric assay after acid hydrolysis. To measure p38 MAPK phosphorylation, the muscle samples were homogenized in lysis buffer ([in mM] HEPES, 150 NaCl, 10 EDTA, 1 Na₃VO₄, 30 NaF, 2 Na₃P₂O with protease inhibitors and 1% Triton X-100) with an OMNI 1005 probe (OMNI International, Warrenton, VA) operating at maximum speed for 20 s and were incubated for 30 min on ice. The samples were spun at 15,000 g for 15 min, and the supernatant was removed and used for analysis. Solubilized protein concentrations were determined by using a bicinchoninic acid protein reagent kit (Pierce Chemical) by using a microtiter plate protocol at 37°C for 30 min. For immunoblotting, aliquot of muscle lysates with equal amounts of protein were separated by SDS-PAGE and transferred to immobil-P polyvinylidine fluoride (PVDF) membranes. The PVDF membranes were blocked with 1% BSA in Tris-buffered saline containing 10 mM Tris, 50 mM NaCl, and 0.05% Tween 20 and incubated overnight with anti-phospho- Thr180/Tyr182-p38 MAPK-specific antibody (New England Biolab no. 9211). Detection was made with a horseradish peroxidase-conjugated secondary antibody (Zymed). The specific band for p38 MAPK was quantified by using a phosphorimager (Molecular Dynamics).

Statistical analysis. Statistical analysis was performed by using a two-way analysis of variance with repeated measures, and Scheffé post hoc comparisons were made when statistical significance was found between observations. Statistical significance was accepted at *P < 0.05. The data reported are means ± SE. The p38 MAPK data are expressed in arbitrary units relative to the basal values in the rested leg.

RESULTS

Three hours after completion of the exercise bout, a bolus injection of insulin was given, and arterial plasma insulin was significantly increased and reached a plateau by 20 min (556 ± 40 pmol/l). Plasma glucose concentration was maintained at 5.2 ± 0.1 mM during the insulin infusion. Whole body glucose infusion rate averaged 54.1 ± 2.9 μmol·min⁻¹·kg⁻¹ during the last 30 min of the clamp. Thigh blood flow was increased (*P < 0.05) similarly in the rested and exercised legs by insulin infusion (Table 1). Insulin-stimulated glucose extraction by the exercised leg was higher (*P < 0.05) compared with the rested leg during the insulin clamp.

Table 1. Blood flow and glucose extraction in rested and exercised leg 3 h after termination of one-legged exercise and during subsequent hyperinsulinemic euglycemic clamp for 100 min

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Leg Blood Flow, l/min</th>
<th>Leg Glucose Extraction, mmol/l*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rested</td>
<td>Exercised</td>
</tr>
<tr>
<td>0</td>
<td>0.23 ± 0.02</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.38 ± 0.04†</td>
<td>0.40 ± 0.06†</td>
</tr>
<tr>
<td>50</td>
<td>0.39 ± 0.05†</td>
<td>0.42 ± 0.07†</td>
</tr>
<tr>
<td>75</td>
<td>0.42 ± 0.06†</td>
<td>0.43 ± 0.07†</td>
</tr>
<tr>
<td>100</td>
<td>0.42 ± 0.06†</td>
<td>0.43 ± 0.05†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Glucose extraction is equal to the arteriovenous difference. Because of technical difficulties with 1 arterial catheter, *n* = 6. †*P < 0.05 vs. 0 min (within leg). ‡*P < 0.05 vs. rested leg.
Thus insulin-stimulated glucose uptake (blood flow × glucose extraction) by the exercised leg was ~50% higher \((P < 0.05)\) compared with the rested leg throughout the insulin clamp (Fig. 1).

Detection of p38 MAPK phosphorylation was made by using a phosphospecific antibody for the sites leading to activation of the enzyme, namely Thr\(^{180}\)/Tyr\(^{182}\). At 3 h after exercise (before insulin infusion), p38 MAPK phosphorylation was ~1.4-fold higher \((P < 0.05)\) in the exercised compared with the rested leg (Fig. 2). Insulin stimulation increased \((P < 0.05)\) p38 MAPK phosphorylation similarly in the rested (~1.2-fold above basal) and exercised muscle (~1.3-fold above basal) at 30 min. The effect of exercise was persistent, as evidenced by higher p38 MAPK phosphorylation in the exercised compared with the rested leg before and during the insulin clamp. However, activation of p38 MAPK by insulin in both the rested and exercised legs reached a maximum by 30 min of the insulin clamp, and no further increases were detectable in either leg at 100 min compared with basal. In neither the rested \((r^2 = 0.17; P = 0.41)\) nor the exercised leg \((r^2 = 0.03; P = 0.74)\) was the absolute increase in glucose transport significantly correlated with the absolute increase in p38 MAPK phosphorylation. The increase in p38 MAPK phosphorylation (fold over basal) tended to be inversely related to muscle glycogen content at 100 min of the insulin clamp (Fig. 3).

**DISCUSSION**

The present results indicate that a high physiological insulin concentration (~600 pmol/l) moderately (20–30%) but significantly stimulates p38 MAPK phosphorylation in human skeletal muscle. This observation underscores the relevance of previous in vitro studies on the insulin effects on p38 MAPK in rat skeletal muscle and cultured myocytes (21, 22). In contrast to the short-lived effect (<8 min) of a supra-physiological insulin injection on p38 phosphorylation in mouse skeletal muscle (9), we now show that the effect under more physiological conditions in humans is more stable and is sustained for at least 100 min. Our results do not permit the conclusion of whether the
insulin-induced p38 MAPK phosphorylation is involved in muscle glucose uptake stimulation. The individual glucose uptake rates did not correlate with p38 MAPK phosphorylation. Still, when the previously exercised leg and the contralateral rested leg are compared, both insulin-induced glucose uptake rate (+55% at 30 min) and p38 MAPK phosphorylation (+45% at 30 min) were significantly higher in the exercised compared with the rested leg ≥3 h after cessation of exercise. None of the presently identified enzymes thought to be involved in the intracellular insulin signal leading to muscle glucose transport are increased by previous exercise in humans; insulin receptor tyrosine kinase activity, insulin receptor substrate-1 phosphorylation, Akt phosphorylation, and glycogen synthase kinase-3 activity are equally altered by insulin in previously exercised compared with nonexercised muscles (24, 26, 27), and phosphatidylinositol-3 kinase activity is even reduced when insulin stimulation is preceded by an exercise bout 3–4 h previously (27). The higher p38 phosphorylation in previously exercised muscles, as shown in the present study, is an attractive putative candidate for explaining the increased insulin sensitivity after exercise, because evidence has been provided that indicates that the enzyme is involved in fully activating GLUT-4 that is present at the surface membrane and not in recruiting the GLUT-4 to the surface membrane (23). Thus the effect on glucose uptake of the presently shown persistent p38 MAPK activation in the hours during recovery from exercise is expected to be low in the basal state (when little GLUT-4 is present at the surface membrane), yet it is expected to be amplified in the insulin-stimulated state (when GLUT-4 content increases at the surface membrane). Thus the effects on glucose uptake are expected to be more apparent in the presence of insulin, as shown in the present study (Fig. 1). In other words, the effect of persistent postexercise p38 activation on glucose uptake is not specific for the insulin stimulus, yet it is far more visible in the insulin-stimulated state. However, future studies are needed to identify whether increased insulin sensitivity after exercise results from a potentiation of a p38 MAPK-dependent pathway leading to GLUT-4 activation. Hansen et al. (11) have previously shown in rats that enhanced insulin sensitivity after exercise is mediated through increased GLUT-4 translocation as evaluated by means of photolabeling of cell-surface GLUT-4. At first glance, these results seem to contradict the above hypothesis that insulin-induced GLUT-4 activation rather than translocation is enhanced by prior exercise. However, it remains to be established whether the bis-mannose photolabeling technique is able to distinguish translocation from activation, because it could be that the photolabel only binds the fully activated GLUT-4 at the sarcolemma.

Recently, Yu et al. (29) found that p38 phosphorylation in human muscle after intense cycling exercise tended to be less pronounced in trained vs. untrained subjects. However, it remains to be determined whether this is causally related to lower glycogen levels in untrained subjects. Several studies have indicated that enhancement of insulin sensitivity after exercise is related to the degree of glycogen depletion in the muscle resulting from the exercise bout (1, 3, 8). The effect of varying glycogen levels on the ability of insulin to activate the phosphatidylinositol 3-kinase-dependent signaling pathway has been investigated (5, 14), whereas the effect on MAPK pathways such as the one involving p38 remains unexplored. In the present study, we found a weak inverse correlation (r = −0.49; P = 0.07) between muscle glycogen content and the degree of p38 MAPK phosphorylation induced by insulin. Because causality cannot be judged from this result, we suggest that a possible role of muscle glycogen content on the insulin stimulation of MAPK pathways should be investigated in a more direct way.

Four isoforms of p38 MAPK (α, β, δ, γ) have been identified in mammalian tissues, including skeletal muscle (10, 12, 13, 17), of which p38γ mRNA levels are highest in this tissue in humans (17). Marathon running activates p38γ phosphorylation (fourfold) but not p38α in human skeletal muscle (2), whereas contractions in isolated rat skeletal muscle induce both p38α and p38β activation (22). Insulin has been shown to activate p38α and p38β isofoms in rat skeletal muscle (22) and in L6 cells (21, 23). In the present study, p38 MAPK phosphorylation was quantified by using a nonisoform-specific antibody; thus we are unable to comment on the specific isoform(s) activated by insulin and by exercise. Further studies would be necessary to ascertain the isoform specificity of p38 MAPK activation by these stimuli in human skeletal muscle.

In summary, we have found that physiological hyperinsulinemia results in a modest yet sustained increase in p38 MAPK phosphorylation in human skeletal muscle. We have also shown that the exercise-induced activation of p38 MAPK phosphorylation is sustained for at least 3 h after exercise and is further activated in response to insulin. Because p38 MAPK seems to be involved in GLUT-4 activation at the cell surface, we raise the hypothesis that this could be related to the enhanced insulin sensitivity observed in skeletal muscle in the period after an exercise bout.

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