Regulation of MAP kinase pathway activity in vivo in human skeletal muscle

ABDULLAH A. OSMAN, MERRI PENDERGRASS, JANICE KOVAL, KATSUMI MAEZONO, KENNETH CUSI, THONGCHAI PRATIPANAWAT, and LAWRENCE J. MANDARINO

Department of Medicine and Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Insulin and exercise potently stimulate glucose metabolism and gene transcription in vivo in skeletal muscle. A single bout of exercise increases the rate of insulin-stimulated glucose uptake and metabolism in skeletal muscle in the postexercise period. The nature of the intracellular signaling mechanisms that control responses to exercise is not known. In mammalian tissues, numerous reports have established the existence of the mitogen-activated protein (MAP) kinase signaling pathway that is activated by a variety of growth factors and hormones. This study was undertaken to determine how a single bout of exercise and physiological hyperinsulinemia activate the MAP kinase pathway. The euglycemic-hyperinsulinemic clamp and cycle ergometer exercise techniques combined with percutaneous muscle biopsies were used to answer this question. In healthy subjects, within 30 min, insulin significantly increased MAP kinase [isoforms p42MAPK and p44MAPK (ERK1 and ERK2)] phosphorylation (141 ± 2%, P < 0.05) and activity (177 ± 5%, P < 0.05), and the activity of its upstream activator MEK1 (161 ± 16%, P < 0.05). Insulin also increased the activity of the MAP kinase downstream substrate, the p90 ribosomal S6 kinase 2 (RSK2) almost twofold (198 ± 45%, P < 0.05). In contrast, a single 30-min bout of moderate-intensity exercise had no effect on the MAP kinase pathway activation from MEK to RSK2 in muscle of healthy subjects. However, 60 min of exercise did increase extracellular signal-related kinase activity. Therefore, despite similar effects on glucose metabolism after 30 min, insulin and exercise regulate the MAP kinase pathway differently. Insulin more rapidly activates the MAP kinase pathway.

INSULIN AND EXERCISE STIMULATE glucose metabolism and gene transcription in vivo in skeletal muscle. In vivo, insulin promptly induces glucose transporter (GLUT-4) translocation to the sarcolemma and increases glucose transport and glucose phosphorylation (5, 11), alters hexokinase activity or subcellular redistribution (43), and increases glycogen synthesis and glycogen synthase activity (4, 23, 42) and pyruvate dehydrogenase and glucose oxidation (30). Insulin also increases the expression of a variety of genes. Likewise, exercise increases muscle glucose transport and glycogen synthase activity and alters gene expression (12, 31, 34).

Insulin produces its effects by binding to the insulin receptor and starting a cascade of events that begins with activation of the insulin receptor β-subunit tyrosine kinase activity, which first phosphorylates the β-subunit of the insulin receptor itself. Tyrosine phosphorylation sites on the receptor serve as recognition motifs for the association of a variety of proteins with the insulin receptor, including insulin receptor sub- strate-1 (IRS-1) (41). IRS-1, for example, is phosphorylated in turn by the receptor tyrosine kinase and serves as a key docking protein for a variety of other proteins, including the regulatory subunits of phosphatidylinositol 3-kinase (PI 3-kinase) (17, 39). PI 3-kinase mediates most of the metabolic effects of insulin and its downstream signaling events (22, 38). Shc and Grb2/Sos link insulin receptor signal- ing to activation of the mitogen-activated protein (MAP) kinase (MAPK) isoenzyme p42MAPK/p44MAPK (ERK1/ERK2) cascade, which is not required for insulin’s metabolic effects but mediates mitogenic signaling (26). MAP kinase pathway activity is increased when Grb2/Sos is recruited to the plasma membrane, where Sos increases the rate of exchange of GTP for GDP on Ras, which in turn activates Raf (MAP/ERK kinase). This leads to activation of MEK, a serine/threonyltyrosine kinase that phosphorylates and activates ERK1 and ERK2. ERK2 is the predominant isoform in skeletal muscle. One of the downstream elements phosphorylated by extracellular signal-related kinases (ERKs) is p90 ribosomal S6 kinase (RSK2). The signaling mechanisms by which exercise alters glucose metabolism and gene expression are less well characterized. Muscle contraction and voluntary exercise increase MAP kinase activity in rodents (19) and human volunteers (1), and it is possible that this pathway is involved in some of the metabolic or gene expression effects of exercise.

Because insulin and muscle contraction bring about many of the same metabolic effects, some investigators have speculated that exercise might share some common signaling elements with insulin receptor signaling (19, 20). However, in rats, neither tetanic contraction nor voluntary wheel running activated insulin receptor signaling (19). In a like manner, vigorous exercise in...
healthy young volunteers did not increase the magnitude of subsequent insulin stimulation of insulin receptor signaling, but it did increase the tyrosine phosphorylation of IRS-1 and the rate of activation of IRS-1-associated PI 3-kinase and glucose clearance (45). These studies suggest that insulin and exercise make use of distinctively different signaling mechanisms to activate glucose metabolism and gene expression.

Many of the studies that have characterized insulin receptor signaling elements have been performed in vitro (9, 13, 16, 17, 32), whereas exercise signaling has been studied mostly in vivo. Several investigations have addressed questions of insulin receptor signaling in vivo in humans, however (1, 8, 45). Hyperinsulinemia during a euglycemic clamp experiment increased insulin receptor tyrosine kinase activity, IRS-1 tyrosine phosphorylation, and the association of PI 3-kinase activity with IRS-1 (45). In humans, the results of another study show that cycle exercise increased MAP kinase (ERK) activity. The present study was undertaken to compare the effects of physiological hyperinsulinemia with those of moderate exercise on systemic glucose disposal, muscle glycogen synthase activity, and MAP kinase signaling activity. Specifically, in the present study, the effects of physiological hyperinsulinemia during a euglycemic clamp were compared with those of moderate exercise (~60% of \( V\dot{O}_2\text{max} \)). Systemic glucose disposal was measured using tritiated glucose, and percutaneous muscle biopsies were performed before and at the end of 30 min of insulin or exercise to assay glycogen synthase activity, MEK1, ERK activity and phosphorylation, and RSK2 activity.

**METHODS AND MATERIALS**

Subjects. A total of 19 healthy volunteers participated in these studies; their characteristics are given in Table 1. Seven subjects participated in the insulin study, and twelve took part in the exercise study. All subjects had normal glucose tolerance, were healthy, and were not taking medications known to affect glucose metabolism. Subjects were instructed not to engage in exercise for 48 h before being studied. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved the protocol, and each subject gave written consent before participating. Insulin receptor signaling through the PI 3-kinase pathway has been characterized in human skeletal muscle. A group of 10 healthy subjects was studied. On the first day, subjects underwent a cycle ergometer determination of maximal \( O_2 \) consumption (\( V\dot{O}_2\text{max} \)), and their anaerobic thresholds were determined by the V-slope method (3). On a separate day, \( \approx 1 \) wk after the \( V\dot{O}_2\text{max} \) test, subjects reported to the GCRC at 0800 having consumed nothing but water since the previous evening. An antecubital vein was catheterized, and [\( ^{3}H \)]glucose was infused in a primed (25 µCi) continuous (0.25 µCi/min) manner. After subjects rested for 30–60 min, a second percutaneous muscle biopsy was performed at \( 60\% \) of \( V\dot{O}_2\text{max} \). Arterialized blood samples were drawn as described for determination of tritiated glucose-specific activities and plasma insulin concentrations. At the end of 30 min of exercise, subjects immediately had a second muscle biopsy in the opposite leg. Muscle samples were rapidly frozen in liquid nitrogen until analysis. The tritiated glucose infusion was ended, and the subject was allowed to leave the GCRC. Two additional healthy control subjects received muscle biopsies before and after 60 min of cycle ergometer exercise at 60% of \( V\dot{O}_2\text{max} \).

Study design. The study consisted of two experimental protocols. Protocol 1 was performed to determine the extent of activation of ERK1/ERK2 by insulin in vivo in skeletal muscle of healthy lean subjects. A group of seven healthy lean subjects was studied. Subjects reported to the General Clinical Research Center (GCRC) at 0800. An antecubital vein was catheterized with a 20-gauge catheter for infusion of insulin and glucose. A primed (25 µCi) continuous infusion of [\( ^{3}H \)]glucose (0.25 µCi/min) was started, and 2 h were allowed for isotopic equilibration. After subjects had rested for 60 min in bed, percutaneous biopsy of the vastus lateralis muscle was performed under local anesthesia (29). The subject was allowed to remain in bed for another 60 min to allow washout of any catecholamine effects of the muscle biopsy. Immediately after the muscle biopsy, euglycemic hyperinsulinemic clamps were performed as previously described (14). An insulin infusion (40 mU·m\(^{-2}\)·min\(^{-1}\)) was started, arterialized blood glucose concentrations were measured every 5 min, and blood glucose was clamped at 5 mM with an infusion of 20% dextrose through the antecubital vein. After 30 min of insulin infusion, a second percutaneous muscle biopsy was taken from the opposite leg, and the insulin infusion was then stopped. All muscle biopsy specimens, which ranged in weight from 75 to 200 mg, were rapidly blotted free of blood and frozen in liquid nitrogen in a cell freezer until analysis. At the end of the study after insulin was stopped, to prevent hypoglycemia, the subject was given a meal, and glucose was infused until the normal plasma glucose concentration was maintained. Activation of glycogen synthase served as a biochemical marker for insulin’s effects.

Protocol 2 was performed to determine how exercise alters phosphorylation and activation of MAP kinase pathways in human skeletal muscle. A group of 10 healthy subjects was studied. On the first day, subjects underwent a cycle ergometer determination of maximal \( O_2 \) consumption (\( V\dot{O}_2\text{max} \)), and their anaerobic thresholds were determined by the V-slope method (3). On a separate day, \( \approx 1 \) wk after the \( V\dot{O}_2\text{max} \) test, subjects reported to the GCRC at 0800 having consumed nothing but water since the previous evening. An antecubital vein was catheterized, and [\( ^{3}H \)]glucose was infused in a primed (25 µCi) continuous (0.25 µCi/min) manner. After subjects rested for 30–60 min, a second percutaneous biopsy of the vastus lateralis muscle was performed, and immediately afterward a hand vein was catheterized retrogradely and placed in a warm (55°C) Plexiglas box to arterialize venous blood for determining tritiated glucose specific activities. The subjects rested for another 30 min and then began cycle exercise for 30 min at a workload that was equivalent to 90% of their anaerobic threshold (~60% of \( V\dot{O}_2\text{max} \)). Arterialized blood samples were drawn as described for determination of tritiated glucose-specific activities and plasma insulin concentrations. At the end of 30 min of exercise, subjects immediately had a second muscle biopsy in the opposite leg. Muscle samples were rapidly frozen in liquid nitrogen until analysis. The tritiated glucose infusion was ended, and the subject was allowed to leave the GCRC. Two additional healthy control subjects received muscle biopsies before and after 60 min of cycle ergometer exercise at 60% of \( V\dot{O}_2\text{max} \).

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Gender (F/M)</th>
<th>BMI (kg/m(^2))</th>
<th>Fasting Plasma Glucose (mM)</th>
<th>Fasting Plasma Insulin (mU/l)</th>
<th>( V\dot{O}_2\text{max} ) (mL·kg(^{-1})·min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>34±5</td>
<td>6/1</td>
<td>25±1</td>
<td>5.1±0.1</td>
<td>7±1</td>
<td>ND</td>
</tr>
<tr>
<td>37±4</td>
<td>6/4</td>
<td>26±1</td>
<td>5.3±0.1</td>
<td>10±2</td>
<td>33.0±4.4</td>
</tr>
</tbody>
</table>

Data are given as means ± SE. ND, not determined; BMI, body mass index.
MEK1 monoclonal antibody was obtained from Transduction Laboratories (Lexington, KY). [32P]ATP was purchased from NEN Life Science Products (Boston, MA). Protein A-agarose, protein G-agarose, myelin basic protein (MBP), and all other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Muscle processing. Muscle biopsies were homogenized in ice-cold lysis buffer containing 50 mM HEPES (pH 7.6), 150 mM NaCl, 2 mM EDTA, 10% glycerol, 20 mM β-glycerophosphate, 20 mM sodium pyrophosphate, 10 mM sodium fluoride (NaF), 2 mM sodium orthovanadate (Na3VO4), 1 mM CaCl2, 1 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml leupeptin, 10 µg/ml aprotinin, and 1% NP-40. Homogenates were centrifuged at 15,000 g for 1 h at 4°C, and muscle debris was removed. Protein concentrations in crude homogenates were estimated by the Lowry method (27). The supernatant was stored at −80°C until used.

Western blotting. Muscle protein (250 µg) was solubilized in SDS sample buffer, boiled for 5 min, loaded onto a 10% SDS-polyacrylamide gel, subjected to electrophoresis, and transferred to nitrocellulose membranes. The membranes were then blocked in TBST (20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) containing 5% nonfat dried milk for 1 h at room temperature. The membranes were then washed three times (5 min each) in TBST and incubated with goat anti-rabbit coupled to horseradish peroxidase; Amersham, Arlington Heights, IL) in TBST in a dilution of 1:1,500 and incubated for 1 h at room temperature. The membranes were then washed three times in TBST and developed with the chemiluminescence detection system according to the manufacturer’s protocol (Amersham). The autoradiographs were subjected to scanning densitometry, and the densities of products were quantified by an imaging densitometer fitted with Molecular Analyst Software.

Kinase assays. ERK2 activity assays were performed as described previously (35). An aliquot of human muscle protein (250 µg) was incubated with 10 µl of anti-murine ERK2 polyclonal antibody at 4°C overnight and adsorbed to 80 µl of 50% slurry of protein A-agarose beads for an additional 2 h. The immune complexes were washed three times with ice-cold lysis buffer and twice with kinase reaction buffer (25 mM HEPES, pH 7.5, 10 mM MgCl2, 2 mM DTT) at 4°C overnight with rabbit polyclonal phosphospecific ERK1/ERK2 antibody (recognizes both phosphorylated ERK1 and ERK2) at a 1:200 dilution in blocking buffer. After being washed three times (5 min each) in TBST, the membranes were incubated with secondary antibody (goat anti-rabbit coupled to horseradish peroxidase; Amersham, Arlington Heights, IL) in TBST in a dilution of 1:1,500 and incubated for an additional 1 h at room temperature. The membranes were then washed three times in TBST and developed with the enhanced chemiluminescence detection system according to the manufacturer’s protocol (Amersham). The autoradiographs were subjected to scanning densitometry, and the densities of products were quantified by an imaging densitometer fitted with Molecular Analyst Software.

RESULTS

Glycogen synthase assay. A portion of the muscle biopsy specimen was homogenized in a buffer consisting of 50 mM potassium phosphate, pH 7.4; 2 mM DTT; 2 mM EDTA; 20 mM NaF; 10 µg/ml leupeptin; 10 µg/ml soybean trypsin inhibitor; 20 µg/ml p-aminobenzamidine; 70 µg/ml N-α-p-tosyl-L-lysine chloromethyl ketone; and 170 µg/ml PMSF. The homogenates were centrifuged at 13,000 g for 10 min. After centrifugation, aliquots of the supernatant (20 µl) were spotted onto P-81 phosphocellulose paper discs in duplicate and washed four times in 0.75% H3PO4 for ≈10 min each. The paper discs were washed once with acetone, air dried, and subjected to scintillation counting.

Whole body glucose disposal and glycogen synthase activity. The effect of exercise (all 10 subjects) and insulin (5 of 7 subjects) on glucose disposal and glycogen synthase activity has been reported previously (24). Insulin increased the rate of glucose disposal from 2.05 ± 0.27 basally to 4.6 ± 1.2 mg·kg−1·min−1 after 120 min (P < 0.05). Exercise also increased glucose disposal from 1.87 ± 0.08 basally to 2.81 ± 0.30 mg·kg−1·min−1 (P < 0.01) after 30 min, but this effect was significantly less than that of insulin. In contrast, exercise increased glycogen synthase activity (GS) more than that of insulin.
than did insulin. GS0.1 increased from 0.27 ± 0.03 to 0.66 ± 0.10 nmol·min⁻¹·mg protein⁻¹ after 30 min of exercise (P < 0.01) but from 0.33 ± 0.07 to 0.47 ± 0.12 nmol·min⁻¹·mg protein⁻¹ after insulin (P < 0.05).

Stimulation of MAP kinase signaling components by insulin and exercise in human muscle biopsies. To determine the extent of insulin- and exercise-induced phosphorylation of ERK1/ERK2 in vivo in normal human skeletal muscle, we first obtained muscle biopsies from healthy subjects immediately before and after 30 min of insulin infusion or moderate exercise. Aliquots of muscle protein were separated by 12% SDS-PAGE and immunoblotted with a phosphospecific ERK1/ERK2 antibody that recognizes only the dual-phosphorylated 42 and 44 kDa ERK proteins. The phosphorylated bands visualized by the ECL system on the immunoblots were quantified by scanning densitometry. The results are shown in Fig. 1A. ERK2 (the most abundant isoform in human muscle) phosphorylation increased by 30 min of insulin infusion (141 ± 2%, P < 0.05). In contrast, 30 min of moderate exercise had no effect on ERK1/ERK2 phosphorylation (Fig. 1B). To examine whether insulin-induced ERK phosphorylation is accompanied by an increase in its kinase activity, we performed an ERK kinase activity assay using the substrate MBP in anti-immunoprecipitates of muscle samples taken from all subjects. Insulin significantly increased ERK2 activity (177 ± 5%, P < 0.05; Fig. 2A). The ability of anti-ERK2 immunoprecipitates to increase 32P incorporation into MBP before and after exercise was also measured (Fig. 2B). The results showed that 30 min of exercise did not increase ERK2 activity above basal level. To determine whether exercise of greater duration increased ERK2 activity, two subjects had muscle biopsies before and after 60 min of exercise, and ERK phosphorylation and activity were assayed as described above. Sixty minutes of exercise increased ERK phosphorylation and activity in both of these subjects (Fig. 3). ERK phosphorylation normalized to ERK2 protein content averaged 0.465 before and 0.921 density units/unit protein after exercise. This was similar to the effect of 30 min of insulin on ERK phosphorylation.

Phosphorylation and activation of MEK are required for activation of ERK1/ERK2 (47). However, under certain conditions, the ERK1/ERK2 and RSK activities may not follow their upstream activator MEK. In many cell types, this loss of correlation may be due to changes in phosphatase activity (7). Six isoforms of MEK are expressed in rodent and human skeletal muscle (21). However, regardless of the species, only MEK1 and MEK2 activate ERK1/ERK2 (21). Therefore, we determined whether insulin or exercise increases MEK activity in human skeletal muscle. MEK1 activity was assayed in muscle protein by measuring the ability of MEK1 immune complexes to phosphorylate a recombinant kinase-inactive MAPK (p42MAPK). The results shown in Fig. 4, A and B, demonstrate that 30 min of insulin infusion significantly increased MEK1 activity above basal level in healthy subjects (161 ± 16%, P < 0.05), but exercise had no effect.

RSK2 is a specific p90 RSK isoform that is phosphorylated and activated by MAP kinase in vitro in response to insulin (10). To examine whether insulin and exercise stimulate RSK2 activation, aliquots of muscle proteins were immunoprecipitated with anti-RSK2 anti-
body, and RSK2 activity was measured in immunoprecipitates using the 3R S6 peptide as substrate. As shown in Fig. 5A, insulin significantly increased RSK2 activity above the basal level (198 ± 6P, 0.05); however, exercise did not activate RSK2 (Fig. 5B).

DISCUSSION

Exercise and insulin both increase glucose transporter translocation, glucose transport, and glycogen synthase activity, and stimulate systemic glucose disposal in vivo in humans (5, 11, 15, 33, 34). Because the effects of insulin and exercise are so similar, it has been theorized that there are common elements in the pathways that signal the responses to these stimuli. Since more details of the insulin receptor signaling system have become known, some investigators have hypothesized that exercise might use some of the elements of the insulin signaling pathway. Goodyear et al. (19) used electrically stimulated muscle contraction and insulin injection, as well as voluntary running, to address this question in rats. Insulin injection rapidly increased muscle insulin receptor tyrosine phosphorylation and activated PI 3-kinase, but muscle contraction did not affect these events (19). Goodyear et al. (18) also examined MAP kinase pathway signaling stimulation by treadmill running and insulin in rats. Exercise increased ERK1/ERK2 phosphorylation, JNK (c-jun NH2-terminal kinase) activity, and RSK-2 activity within 10 min, and this increase was sustained for ≥60 min (19). Insulin also increased ERK phosphorylation and RSK-2 activity, but not JNK activity (19). Two studies in humans reported that exercise increases MAP kinase pathway (ERK) activity. Aronson et al. (2) showed that 60 min of exercise at a workload corresponding to 70% VO2max increased ERK phosphorylation and RSK-2 activity, as well as Raf-1, MEK1, and RSK2 activities. Widegren et al. (44) showed that 30 min of exercise activated ERK, stress-activated protein kinase/ERK kinase 1, and p38 MAP kinase, but not protein kinase B (PKB). One-legged exercise was also used in those studies to demonstrate that the effect of exercise on ERK phosphorylation was local rather than systemic (2). However, activation of p38 MAP kinase also occurred in the rested leg (44). Other recent studies also failed to find an effect of muscle contraction on signaling mediated through PKB (6, 28).

The present study was undertaken to compare how insulin and exercise activate the MAP kinase signaling pathway in humans. To compare these stimuli, an
attempt was made to make them physiologically similar. The same duration, 30 min, was used for both. The insulin infusion produced plasma insulin concentrations well within the physiological range. The exercise was moderate in intensity (60% of $V\dot{O}_{2\text{max}}$) and was chosen to mimic the intensity and duration of an exercise bout that would be typical for the average nonathlete. Although different subjects took part in the insulin and exercise protocols, the two groups were similar with regard to gender composition, body mass index, and age, and no subject in any group engaged in any regular exercise. Therefore, these groups could be expected to have similar responses to either exercise or insulin, and it is unlikely that the difference between activation of the MAP kinase pathway with insulin vs. exercise is due to differences between subjects. Despite this, 30 min of exercise did not activate the MAP kinase pathway, but 30 min of insulin significantly increased MEK, ERK, and RSK2 activity. However, when the duration of exercise was increased to 60 min, ERK activity was approximately doubled. This confirms the results of previous studies (2). We conclude from these results that insulin more acutely increases the MAP kinase pathway than does exercise.

It is also conceivable that the ability of exercise to stimulate MAP kinase activity after 60 min is due to increased sensitivity to low insulin concentrations that may be sufficient to produce an increase in ERK activity and phosphorylation. Although theoretically possible, the plasma insulin concentrations after 60 min of exercise are unlikely to have been high enough to produce any activation of MAP kinase pathway activity. Although they were not measured in this study, it is likely that the plasma insulin concentrations fell during exercise (24).

The present results also show that exercise-induced activation of glucose uptake and metabolism are not dependent upon activation of the MAP kinase pathway. This is consistent with the results of recent studies in rodents (46). However, it still could have been hypothesized that changes in gene expression brought about by exercise were mediated by the MAP kinase pathway. Nevertheless, at least with a single 30-min bout of exercise, the MAP kinase pathway was not activated, so it could not be responsible for exercise-induced

Fig. 4. Effect of insulin (A) and exercise (B) on MEK1 activation in human skeletal muscle in vivo. Aliquots of muscle protein (300 µg) from healthy subjects ($n = 6$) were obtained and immunoprecipitated, and MEK1 activity was measured on basis of phosphorylation of glutathione-S-transferase (GST)-ERK2, as described in METHODS. A representative gel of 62-kDa band corresponding to phosphorylated GST-ERK2 developed by PhosphorImager is shown above graph. Intensity of phosphorylation of kinase-inactive GST-ERK2 was quantified by PhosphorImager analysis to determine relative MEK1 activity. Values are means ± SE of PhosphorImager relative density units. * $P < 0.05$ vs. basal values. No statistically significant difference was observed between basal and postexercise values in muscle samples studied ($P > 0.05$).

Fig. 5. Effect of insulin (A) and exercise (B) on ribosomal S6 kinase 2 (RSK2) activation in vivo in human skeletal muscle. Aliquots of muscle proteins (400 µg) from healthy subjects ($n = 6$) were obtained and immunoprecipitated with anti-RSK2 antibody. RSK2 activity was measured in immunoprecipitates with 3R S6 peptide as substrate, as described in METHODS. $^{32}$P incorporated into the substrate (in cpm) were measured by liquid scintillation counting. Values are means ± SE. * $P < 0.05$ vs. basal values. No statistically significant difference was observed between basal and postexercise values in muscle samples studied ($P > 0.05$).
changes in gene expression. However, the time response for activation of the MAP kinase pathway by exercise may have implications for any training responses that might be mediated by the MAP kinase pathway. It could be predicted from these results that for responses that depended upon MAP kinase pathway activation, an exercise duration >30 min/session might be required. These results provide further evidence that, even though insulin and exercise produce many of the same metabolic and gene expression events in skeletal muscle, they do not share the same proximal signaling pathways. Presumably, downstream convergence points in these signaling pathways exist, but they remain to be discovered.

These results show that some of the distal metabolic effects of these two stimuli are similar in direction but differ qualitatively. For instance, both stimuli increase glucose disposal, but within 30 min, insulin is more effective at this. Both increase glycogen synthase activity, but exercise is more effective than insulin in this respect. The lack of correspondence between the magnitude of the effects of insulin and exercise on glycogen synthase activity and glucose disposal suggests that a given increase in the activity of this enzyme is not always sufficient to produce the same magnitude of increase in glucose disposal. This may bear on the question of whether glycogen synthase is a rate-determining step in glucose uptake (36). Although overexpression of glycogen synthase in mice increases glucose uptake in skeletal muscle (25), studies using NMR spectroscopy have led to the conclusion that glucose transport, rather than glycogen synthase, experts greater control over glucose disposal (37). These differences may indicate that, under different conditions, both glucose transport and the activity of glycogen synthase may be relatively more or less important for determining the rate of glucose uptake. Another point of note with respect to glycogen synthase is that, although 30 min of exercise did not activate the MAP kinase pathway, glycogen synthase activity was markedly increased. This activation is sustained for long periods (24); therefore, it is unlikely that exercise signals glycogen synthase through the MAP kinase pathway, consistent with recent results in rats (46).

The fact that a physiological concentration of insulin led to MAP kinase activation within 30 min indicates that this is a physiologically relevant phenomenon. The results of in vitro studies suggest that the effects of insulin on glucose metabolism are independent of MAP kinase pathway activity, because they are unaffected by either a dominant negative Ras inhibitor (16) or a pharmacological inhibitor of MEK, PD-098059 (26). Therefore, the physiological role of MAP kinase pathway activation by insulin is unclear at present. However, it is possible that, in muscle in vivo, insulin stimulation of the MAP kinase pathway might mediate the ability of insulin to suppress protein breakdown (40). It is also possible that the MAP kinase pathway can desensitize or “dampen” insulin signaling through the PI 3-kinase pathway. Results of in vitro experiments show that ERK can phosphorylate IRS-1 on serine residues and potentially inhibit this pathway (13).

In summary, the results of this study show that the same duration (30 min) of physiological hyperinsulinemia and moderate exercise has different effects on the ERK1/ERK2 pathway, including the downstream signaling element RSK2. Therefore, the metabolic or gene expression effects of 30 min of exercise cannot be mediated through the MAP kinase pathway in humans in vivo. Activation of the MAP kinase pathway occurs more rapidly in response to insulin than to exercise.

The authors gratefully acknowledge the technical assistance of Andrea Barrentine, Kathy Camp, Gilbert Gomez, J an Finlayson, Cindy Munoz, and Shelia Taylor. The excellent nursing assistance of Patricia Wolff and Norma Ortiz is also acknowledged.

This study was supported by a grant from the American Diabetes Association (L. J. Mandarino), and National Institute of Diabetes and Digestive and Kidney Diseases Grant R01 DK-47936 to L. J. Mandarino and NIH, Division of Research Resources Grant RR-0346 to the General Clinical Research Center, Audie Murphy Veterans Affairs Hospital.

Present address: M. Pendergrass, Dept. of Medicine, Tulane University Medical School, New Orleans, LA; K. Maezono, Ajinomoto Co., Inc., Yokohama, Japan.

Address for reprint requests and other correspondence: L. J. Mandarino, Division of Diabetes, Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7886 (E-mail: mandarino@uthscsa.edu).

Received 12 April 1999; accepted in final form 17 December 1999.

REFERENCES


