Exercise and insulin cause GLUT-4 translocation in human skeletal muscle

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INSULIN AND EXERCISE are the two most physiologically relevant stimulators of glucose uptake in skeletal muscle. Numerous studies using rat skeletal muscle have demonstrated that the major mechanism for the insulin- and exercise-induced increases in glucose uptake involves the translocation of the GLUT-4 glucose transporter isoform from an intracellular location to the cell surface (22, 23). More recently, fractionation procedures originally developed for use in rat skeletal muscle have been adapted for the study of GLUT-4 physiology in human skeletal muscle. Using a subcellular fractionation method that recovers ~10% of plasma membranes from human skeletal muscle, we have shown that ingestion of a standard 75-g oral glucose load results in a significant increase in plasma membrane GLUT-4 protein (18). This study demonstrated that GLUT-4 translocation can occur in response to normal physiological increases in blood glucose and insulin concentrations. Insulin infusion that results in hyperinsulinemia has also been shown to result in GLUT-4 translocation in skeletal muscle of normal subjects in several studies (15, 20, 25, 46, 51), but not in one other study (32). By use of a giant sarcolemmal vesicle preparation, cycle exercise in humans has been reported to increase GLUT-4 in this fraction (28, 29), although this preparation is not sensitive to insulin stimulation (36).

In humans, a single exercise session can increase whole body glucose disposal, with the majority of glucose being taken up by working skeletal muscle (26). The period after exercise is characterized by a substantial increase in insulin-stimulated glucose disposal in human subjects, as determined by the euglycemic-hyperinsulinemic clamp technique (5, 14, 34, 39). This increase in glucose disposal is primarily a function of increases in glucose uptake and nonoxidative glucose disposal in skeletal muscle (14, 39), and studies using one-legged exercise models have demonstrated that the exercise-induced increase in insulin action is a local phenomenon restricted to the exercised muscles (38, 39). It is not known whether the additive effects of exercise and insulin to increase glucose uptake are associated with an enhancement of GLUT-4 translocation in human skeletal muscle.

Molecular signaling mechanisms that may contribute to the increase in insulin sensitivity for glucose uptake in previously exercised skeletal muscle have not been elucidated. However, it is known that prior exercise in human subjects does not enhance insulin activation of proximal steps in insulin signaling, including insulin receptor tyrosine kinase activity, insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation, and IRS-1-associated phosphatidylinositol 3-kinase (PI3-kinase) activity (48). Akt is a serine/threonine kinase that can be activated through both PI 3-kinase-dependent (1, 11, 37) and -independent mechanisms (33, 40, 41) and has been implicated in the regulation of insulin-
stimulated glucose uptake (10, 12, 27, 30, 42, 45). Most (9, 31) but not all (44) studies report that contraction of rat skeletal muscles does not increase Akt activity or phosphorylation. The combined effects of physical exercise and insulin stimulation on this potential mediator of GLUT-4 translocation have not been reported.

In the current investigation we studied GLUT-4 translocation in human skeletal muscle in response to the normal physiological stimuli of exercise and insulin, and we determined whether prior exercise increases insulin-stimulated GLUT-4 translocation to the plasma membrane. Furthermore, we studied the effects of insulin, exercise, and the combination of exercise plus insulin on Akt phosphorylation in human skeletal muscle. Our results demonstrate that moderate-intensity exercise and physiological hyperinsulinemia result in a similar increase in GLUT-4 recruitment to the plasma membrane in human skeletal muscle, and they further suggest that there is a partially additive effect on GLUT-4 translocation with the combination of exercise plus insulin. The increased insulin-stimulated GLUT-4 translocation in the previously exercised skeletal muscle was associated with an increase in insulin-stimulated Akt activity.

METHODS

Subjects

Nine healthy volunteers (7 males and 2 females) were included in the study. The subjects were screened by a health questionnaire and physical examination. Exclusion criteria included any clinical evidence for cardiac, pulmonary, or metabolic abnormalities. To determine the appropriate intensity for the acute bout of exercise, subjects underwent maximal oxygen consumption (V\textsubscript{O}\textsubscript{2max}) testing several days before participation in the study protocol. The physical characteristics of the subjects are shown in Table 1.

Experimental Protocols

The study was approved by the Institutional Ethical Committee at the Karolinska Institute. The subjects were informed of the nature and the purpose of the study, and informed consent was obtained from each subject. The study consisted of two experimental protocols (Fig. 1), both of which were performed after an overnight fast.

Protocol 1. For the first protocol, the effect of physiological hyperinsulinemia (60 µU/ml) for 2 h was investigated (Fig. 1A). Basal blood sampling was performed after a 60-min period of rest in the supine position. At this time, a muscle biopsy (Rest Biopsy) was obtained from the lateral aspect of the quadriceps muscle, as we will describe. After closure of fascia and skin, a constant infusion of insulin was started at a rate of 0.8 mU·kg\textsuperscript{-1}·min\textsuperscript{-1}, and a euglycemic-hyperinsulinemic clamp study was performed as described previously (13). Blood sampling was performed at 30-min intervals during the 2nd h for subsequent analysis of glucose and insulin concentrations. At the end of the 2-h clamp protocol, a final biopsy (Rest Biopsy) was obtained from the lateral aspect of the quadriceps muscle, as we will describe. After closure of fascia and skin, a constant infusion of insulin was started at a rate of 0.8 mU·kg\textsuperscript{-1}·min\textsuperscript{-1}, and a euglycemic-hyperinsulinemic clamp study was performed as described previously (13). Blood sampling was performed at 30-min intervals during the 2nd h for subsequent analysis of glucose and insulin concentrations. At the end of the 2-h clamp protocol, a final biopsy (Rest Biopsy) was obtained through the previous incision. For this second biopsy, the sample was taken from an area of the muscle that was adjacent to but separate from fibers handled in the first biopsy.

Protocol 2. For the second protocol, the effects of exercise and exercise + insulin were investigated (Fig. 1B). This protocol was performed 28 ± 3 days after protocol 1, and none

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value ± SE</th>
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<tbody>
<tr>
<td>Gender</td>
<td>7M/2F</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178 ± 5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 6</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>22.9 ± 0.9</td>
</tr>
<tr>
<td>V\textsubscript{O}\textsubscript{2max}, ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>47.1 ± 1.8</td>
</tr>
<tr>
<td>Basal insulin, µU/ml</td>
<td>10.0 ± 0.6</td>
</tr>
<tr>
<td>Basal glucose, mM</td>
<td>4.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. BMI, body mass index; V\textsubscript{O}\textsubscript{2max}, maximal O\textsubscript{2} consumption.
of the subjects reported discomfort from the leg used in protocol 1. With the subjects in a supine position, the identical site in the contralateral leg (compared with protocol 1) was marked with a surgical pen. Three electrocardiogram electrodes were placed at the chest, and intravenous cannulas were inserted in an antecubital or dorsal hand vein bilaterally for later infusions and blood sampling. The subjects exercised on a cycle ergometer for a total of 60 min at a load corresponding to 70% of their VO\textsubscript{2max}. After 50 min of exercise, the subjects stopped exercise, and 5–15 ml of Citanest were injected in the marked area at the quadriceps muscle for local anesthesia of the skin and subcutaneous tissue. The time elapsed during this interruption was 32 ± 6 s, and the exercise then proceeded for an additional 10 min. After 60 min of exercise, the subjects moved to a bed for basal blood sampling, and a muscle biopsy was obtained (Exercise Biopsy). The time elapsed between cessation of exercise and cutting of muscle fibers was 563 ± 12 s. After closure of fascia and skin, a 2-h euglycemic-hyperinsulinemic clamp was performed exactly as described above, followed by a second muscle biopsy (Exercise + Insulin Biopsy).

**Muscle Biopsies**

The muscle biopsies were obtained from the lateral portion of the quadriceps muscle. A bundle of muscle fibers (~0.8 g) was dissected as previously described in detail (18). A small piece of this muscle sample (~0.1 g) was immediately frozen in liquid nitrogen and used to measure glycogen content according to the method of Hultman (24) and to assess Akt phosphorylation (see Muscle Processing and Immunoblotting for Akt Ser\textsuperscript{473} Phosphorylation). The remaining muscle was rinsed in saline, dissected free of connective tissue and fat, weighed, and used for preparation of skeletal muscle plasma membranes (see Skeletal Muscle Fractionation, Marker Enzyme Analysis, and GLUT-4 Immunoblotting). For the second biopsy of each treatment period, the incision site was opened, and a second bundle of muscle fibers was extracted and treated identically to the first.

**Skeletal Muscle Fractionation, Marker Enzyme Analysis, and GLUT-4 Immunoblotting**

Muscle samples were minced, homogenized at the time of biopsy in a buffer containing 250 mM sucrose and 20 mM HEPES (pH 7.4), frozen in liquid N\textsubscript{2}, and stored at −80°C for up to 4 mo. Plasma membranes were isolated using our procedure that has been adapted for use in human skeletal muscle (18). Protein concentrations in the homogenate and plasma membrane fractions were determined by the Bradford method (7). The plasma membrane marker enzyme, 5'-nucleotidase, was measured in the homogenate and the plasma membrane fraction to determine purity and recovery of plasma membranes (3). Aliquots of homogenate (100 µg) and plasma membrane (10 µg) proteins were immunoblotted for GLUT-4 as previously described (18). Plasma membrane GLUT-4 was expressed relative to 5'-nucleotidase activity to adjust for differences in plasma membrane purity. Compared with preparing plasma membranes from fresh muscle, we have found that the procedure used for the current study and length of storage has no effects on isolation of plasma membranes (purity and recovery of 5'-nucleotidase) or total muscle GLUT-4.

**Muscle Processing and Immunoblotting for Akt Ser\textsuperscript{473} Phosphorylation**

A small portion of the muscle sample that was immediately frozen in liquid N\textsubscript{2} at the time of dissection was processed in a detergent-containing lysis buffer, as previously described (2). To measure Akt phosphorylation, muscle proteins (80 µg) were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in Tris-buffered saline (TBS) plus Na\textsubscript{2} (TNA) containing 5% BSA for 1 h at room temperature. The membranes were incubated overnight at 4°C with a phosphoserine\textsuperscript{473}-specific Akt (2 µg/ml) in TNA containing 5% BSA. Membranes were washed in TBS plus 0.05% Tween-20, incubated with α-rabbit Ig-horseradish peroxidase (HRP; 1:2,000), and antibody binding was detected by enhanced chemiluminescence.

**Materials**

Insulin (Actrapid Human) was from Novo (Bagsvaerd, Denmark). Reagents for SDS-PAGE and protein assays were from Bio-Rad Laboratories (Richmond, CA). Phosphospecific Akt antibody was from New England Biolabs (Beverly, MA), and α-rabbit Ig-HRP was from Amersham (Arlington Heights, IL). DNAse was purchased from Worthington Biochemicals (Freehold, NJ). Glucose (hexokinase) assay reagent for glycogen determinations was purchased from Sigma Chemical (St. Louis, MO). \textsuperscript{125}I-labeled protein A was obtained from ICN Biomedical (Costa Mesa, CA). Other chemicals and reagents were from Fisher Scientific (Lexington, MA) or Sigma Chemical.

**Statistical Analyses**

All values are given as means ± SE. Differences among groups were analyzed using repeated-measures ANOVA, and post hoc analysis was performed by the Newman-Keuls multiple comparison test. Comparisons between insulin and insulin + exercise for Figs. 2B and 4C were done by paired Student\textquotesingle s t-test.

**RESULTS**

**Concentrations of Glucose, Lactate, and Insulin and Insulin Infusion Rates**

Basal and steady-state clamp concentrations of glucose, lactate, and insulin are shown in Table 2. Basal and steady-state clamp glucose concentrations were similar for both protocols. The mean coefficients of variation for blood glucose during the steady-state clamp were 4.6 and 4.8% for protocols 1 and 2, respectively. Exercise resulted in a 3.5-fold increase in blood lactate concentrations, whereas insulin and glucose infusion increased lactate concentrations by 1.5-fold above rest. Although plasma insulin concentrations did not decrease with exercise, there was a significantly lower degree of physiological hyperinsulinemia during the postexercise clamp.

Table 2. Plasma concentrations of glucose, lactate, and insulin at rest, immediately after exercise, or during steady-state insulin infusion

<table>
<thead>
<tr>
<th>Glucose, mmol/l</th>
<th>4.4±0.1</th>
<th>4.4±0.1</th>
<th>4.7±0.2</th>
<th>4.6±0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate, mmol/l</td>
<td>0.53±0.04</td>
<td>0.80±0.05</td>
<td>1.88±0.25*</td>
<td>0.80±0.04</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>10±1</td>
<td>61±5*</td>
<td>14±1</td>
<td>52±2*</td>
</tr>
<tr>
<td>GIR, µg kg\textsuperscript{-1} min\textsuperscript{-1}</td>
<td>6.87±0.60</td>
<td>7.63±0.55</td>
<td></td>
<td></td>
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</tbody>
</table>

Values are means ± SE; n = 9. GIR, glucose infusion rates. *P < 0.001 vs. REST, †P < 0.05 vs. INSULIN.
The glucose infusion rates (GIRs) during insulin infusion for periods 0–30, 30–60, 60–90, and 90–120 min are shown in Fig. 2A. During the postexercise clamp there was a greater requirement for glucose infusion during physiological hyperinsulinemia, and this difference was statistically significant for the periods of 0–30, 30–60, and 60–90 min and for the entire 0–120 min (P < 0.05). In addition, less time was required to achieve steady-state GIR during the postexercise clamp, as the GIR for the 30- to 60-min period was not different from the GIR for the 90- to 120-min period. The GIR was not statistically different between the insulin and exercise + insulin clamps for the final 30-min period (90–120 min), nor was the GIR for the entire steady-state glucose clamp period (60–120 min) different between the two protocols (Table 2). The calculated GIR-to-I ratio (GIR divided by the prevailing plasma insulin concentrations) was significantly higher during the postexercise clamp (Fig. 2B).

Muscle Glycogen Concentrations

In protocol 1, when subjects were studied in the resting condition, 2 h of insulin infusion that resulted in physiological insulin concentrations did not alter glycogen content in the vastus lateralis muscle (Fig. 3).
In protocol 2, 60 min of exercise decreased skeletal muscle glycogen content by 62%. After the 2-h period of insulin infusion, glycogen concentrations were not significantly increased compared with the muscle obtained immediately postexercise and were substantially lower than in the resting state.

**Plasma Membrane Recovery and Enrichment**

Characteristics of the plasma membrane and homogenate fractions are shown in Table 3. To estimate plasma membrane recovery and enrichment in the fraction purified from the muscle biopsy, we measured the activity of 5'-nucleotidase, an enzyme that is predominantly associated with surface membranes (3). 5'-Nucleotidase activity data demonstrate that ~9% of the plasma membranes were recovered from the muscle tissue. This fraction was enriched by ~36- to 45-fold over the activity of 5'-nucleotidase in the starting muscle homogenate.

**Homogenate and Plasma Membrane GLUT-4**

Figure 4A shows representative immunoblots of plasma membrane GLUT-4 from two subjects, and Fig. 4B shows quantitation of all nine subjects. Compared with rest, there was a 32% increase in plasma membrane GLUT-4 in response to physiological hyperinsulinemia. Exercise for 60 min at a workload corresponding to 70% of V\(\text{O}_2\)\(_{\text{max}}\), also resulted in a significant 34% increase in plasma membrane GLUT-4 compared with resting values. The combination of exercise + insulin increased plasma membrane GLUT-4 by 44% above rest, which was not a statistically significant increase above the effects of insulin or exercise alone. However, when the data are expressed relative to the plasma insulin concentration during the steady-state period of the clamp, prior exercise significantly increased plasma membrane GLUT-4 protein compared with insulin treatment alone (Fig. 4C). There was no effect of the insulin, exercise, or exercise + insulin treatments on total muscle homogenate GLUT-4 content (data not shown).

### Akt Ser\(^{473}\) Phosphorylation

Because previous work has shown that proximal steps in insulin signaling (i.e., insulin receptor tyrosine kinase activity, IRS-1 tyrosine phosphorylation, and IRS-1-associated PI 3-kinase activity) are not enhanced in the postexercise state in human skeletal muscle (48), in the current investigation we measured Akt signaling, a molecule downstream of PI 3-kinase in this insulin signaling cascade. To assess Akt activity we used a phosphospecific antibody that only recognizes the protein in its phosphorylated (activated) state (Fig. 5A). In preliminary experiments we have determined that Akt Ser\(^{473}\) phosphorylation closely follows Akt activity in both rat and mouse skeletal muscle (J. F. P. Wojtaszewski, J. F. Markuns, and L. J. Goodyear, unpublished observations). In addition, on the basis of previous findings for upstream signaling elements (48) and Akt (J. F. P. Wojtaszewski, L. J. Goodyear, and E. A. Richter, unpublished observations), we are confident that after 2 h of physiological hyperinsulinemia, the elevated signaling activity reflects a steady-state activity level. Figure 5B shows that insulin increased Akt phosphorylation by 6.4-fold above rest and that this insulin-stimulated increase was further increased in the previously exercised muscle (10.1-fold above rest). Interestingly, Akt phosphorylation was slightly increased with exercise in each individual subject (mean increase = 2.8-fold above rest; \(P < 0.1\)).

**DISCUSSION**

This study demonstrates that an insulin infusion that results in insulin concentrations in the physiological range (~56 \(\mu\)U/ml) causes a significant recruitment of GLUT-4 to the plasma membrane. These findings are in agreement with our previous report, in which oral ingestion of a glucose load with ensuing insulin concen-
trations of ~40 µU/ml increases plasma membrane GLUT-4 content (18), and work from other groups demonstrating that insulin infusion resulting in insulin concentrations of 90–100 µU/ml (20, 51) or ~3,000 µU/ml (15) causes GLUT-4 translocation in human skeletal muscle. Interestingly, direct comparison of these studies reveals that the larger the resultant plasma insulin concentration, the greater the increase in plasma membrane GLUT-4 content divided by the prevailing plasma insulin concentrations during steady-state blood glucose (60–120 min) in 9 healthy volunteers during a 2-h euglycemic-hyperinsulinemic clamp (C). Measurements were performed at rest (INSULIN, open bar) and after 60 min of exercise (EX + INS, filled bar). Data are means ± SE. *P < 0.05 vs. REST. Plasma membrane GLUT-4 divided by the prevailing plasma insulin concentrations during steady-state blood glucose (60–120 min) in 9 healthy volunteers during a 2-h euglycemic-hyperinsulinemic clamp (C). Measurements were performed at rest (INSULIN, open bar) and after 60 min of exercise (EX + INS, filled bar). Data are means ± SE. **P < 0.01 vs. INSULIN.

Fig. 4. Plasma membrane GLUT-4 and ratio of plasma membrane GLUT-4 to steady-state plasma insulin concentration. Representative immunoblots of plasma membrane GLUT-4 from 2 subjects (A) and quantitation of plasma membrane GLUT-4 content expressed relative to 5′-nucleotidase activity from all 9 healthy volunteers (B). Open muscle biopsies were obtained from the vastus lateralis muscle at rest (REST), after a 2-h euglycemic-hyperinsulinemic clamp (INSULIN), after 60 min of exercise at a workload corresponding to 70% of VO2max (EXERCISE), and after exercise followed by a 2-h euglycemic-hyperinsulinemic clamp (EX + INS). Data are means ± SE. *P < 0.05 vs. REST. Plasma membrane GLUT-4 divided by the prevailing plasma insulin concentrations during steady-state blood glucose (60–120 min) in 9 healthy volunteers during a 2-h euglycemic-hyperinsulinemic clamp (C). Measurements were performed at rest (INSULIN, open bar) and after 60 min of exercise (EX + INS, filled bar). Data are means ± SE. **P < 0.01 vs. INSULIN.

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vesicles were isolated by collagenase treatment (28, 29). Nevertheless, using these vastly different methodologies, both groups have demonstrated that physical exercise causes a redistribution of GLUT-4 in human skeletal muscle.

In addition to the insulin-independent effects of exercise to increase glucose uptake in muscle, the period after exercise is typically characterized by an increase in the sensitivity of muscle to stimulation by insulin (reviewed in Ref. 19). We hypothesized that the enhanced insulin-stimulated glucose uptake in human skeletal muscle immediately after exercise is due to an increase in GLUT-4 translocation. Our results show that the combination of exercise followed by insulin stimulation results in only a slightly higher plasma membrane GLUT-4 content compared with the effects of insulin or exercise alone. However, the difference in insulin concentrations during the clamp could make these data difficult to interpret. Increased insulin clearance has been observed in previous studies (8, 35) and can last for as long as 48 h after acute cycle exercise. Therefore, we also presented the data expressed relative to the prevailing insulin concentrations. When these data are expressed relative to the steady-state plasma insulin concentration during the clamp, there was a 25% increase in the plasma membrane GLUT-4-to-insulin ratio when the subjects had exercised before insulin treatment. This was similar to the 25% increase in the GIR-to-insulin ratio during the steady-state period of the clamp after exercise. If we assume, on the basis of some of our rat time course studies (17) and human studies (4) that the effect of exercise per se on GIR and GLUT-4 translocation is rapidly reversed, then the fact that lower plasma insulin concentrations are able to raise GIR and GLUT-4 to similar or slightly greater levels suggests greater sensitivity to insulin after exercise in the skeletal muscle from the human subjects.

One study in rat skeletal muscle has demonstrated that, 3.5 h after a single bout of exercise, submaximally insulin-stimulated GLUT-4 translocation is increased in isolated epitrochlearis muscle (21).

It is also possible that the enhanced GLUT-4 translocation in the postexercise period is due to the prolonged effect of the exercise session per se. The additive effect of exercise on insulin-stimulated GLUT-4 translocation might have been greater if the muscle biopsy had been taken at an earlier time point after the start of insulin infusion. The GLUT-4 recruited to the plasma membrane in response to exercise may have been sustained at the cell surface and then internalized intracellularly over time. This could explain how steady-state GIR was attained so rapidly (30–60 min) in the postexercise clamp, and why the exercise effect was beginning to diminish during the last 30 min of the 2-h clamp (Fig. 2A). Regardless of whether these effects are due to additivity or increased insulin sensitivity, our data provide the first evidence that a mechanism for increased postexercise glucose uptake is due to an enhanced GLUT-4 translocation in human skeletal muscle.

The cellular mechanism leading to the postexercise increase in insulin-stimulated glucose uptake and GLUT-4 translocation in human skeletal muscle could involve the enhancement of insulin receptor signaling. Although we did not measure proximal insulin-signaling molecules in the current study because of a lack of adequate sample, previous studies have demonstrated that exercise does not change insulin binding to its receptor (6, 43, 52) and that prior exercise does not increase insulin-stimulated receptor tyrosine kinase activity in skeletal muscles obtained from rats (43) or humans (48). Furthermore, insulin's ability to activate IRS-1-associated PI 3-kinase activity in vivo is diminished in previously exercised human muscle (48). In contrast, in rat skeletal muscle, one report has demonstrated that prior exercise increases insulin-stimulated PI 3-kinase activity in phosphotyrosine immunoprecipitates (50). In the current investigation, we measured the phosphorylation of Akt, a protein downstream of PI 3-kinase that may function in the regulation of insulin-sensitive glucose transport in muscle (10, 12, 27, 30, 42, 45). Our findings showing enhanced insulin-stimulated Akt phosphorylation in previously exercised skeletal muscle are quite intriguing, and they raise the possibility that this more distal step in the insulin-signaling cascade plays a role in the postexercise increase in insulin-stimulated GLUT-4 translocation. Consistent with these findings in human skeletal muscle, we have recently observed that prior exercise also increases insulin-stimulated Akt phosphorylation and activity in mouse skeletal muscle (J. F. P. Wojtaszewski and L. J.).

**Fig. 5. Akt Ser473 phosphorylation.** Representative immunoblot of Akt phosphorylation (AKT-P) from 1 subject (A) and quantitation of Akt phosphorylation (Phospho-AKT) from 7 healthy volunteers (B). Open muscle biopsies were obtained from the vastus lateralis muscle at rest (REST), after a 2-h euglycemic-hyperinsulinemic clamp (INSULIN), after 60 min of exercise at a workload corresponding to 70% of $V_{O2_{max}}$ (EXERCISE), and after exercise followed by a 2-h euglycemic-hyperinsulinemic clamp (EX + INS). Data are means ± SE. **P < 0.001 vs. REST; #P < 0.001 vs. INSULIN.
Goodyear, unpublished observation). Clearly, Akt as a regulator of the exercise-induced enhancement of insulin-stimulated GLUT-4 translocation in human skeletal muscle will be an important area for future study.

In addition to the effects of prior exercise to increase insulin-stimulated Akt phosphorylation, in the current study the 60-min exercise session slightly increased Akt phosphorylation. Previous studies, including our own work, have suggested that contraction of isolated rat skeletal muscles in vitro in the absence of insulin (9, 31), contraction of rat hindlimbs in situ via electrical stimulation (J. F. Markuns and L. J. Goodyear, unpublished observation), and cycle exercise in human subjects (47) do not increase Akt activity/phosphorylation. Interestingly, one recent report has shown that contraction of hindlimb muscles by electrical stimulation does not increase Akt2 or Akt3 activity but increases Akt1 activity by approximately threefold (44). This magnitude of increase in Akt1 activity is very similar to the increase in Akt phosphorylation observed in the current investigation (2.8-fold), and it is noteworthy that the phospho-specific antibody used in our study was made against the Akt1 sequence (although presumably it also recognizes the other Akt isoforms). The mechanism leading to the small increase in Akt activity with exercise is unlikely to involve PI 3-kinase, because there is considerable evidence that muscle contraction and exercise do not increase PI 3-kinase activity (16, 49, 50). PI 3-kinase-independent mechanisms have been described for the activation of Akt (33, 40, 41), and perhaps exercise is working through a similar type of signaling system. Defining the molecular mechanisms leading to the exercise-induced increase in Akt activity, and determining whether there is physiological significance to this small activation of the enzyme, will also be an important focus for future investigation.

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-42238 and AR-45670 (L. J. Goodyear), a grant from the Boehringer Mannheim Corporation (E. S. Horton and L. J. Goodyear), and grants from the Karolinska Institute, the Swedish Medical Research Council (no. 09101), the Swedish Diabetes Association, the Fredrick and Ingrid Thürings Foundation, the Swedish Society of Medicine, and Nutricia, AS, the Netherlands.

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Received 29 January 1999; accepted in final form 4 June 1999.

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