Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in human skeletal muscle

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Houmard, Joseph A., Christopher D. Shaw, Matthew S. Hickey, and Charles J. Tanner. Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in human skeletal muscle. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E1055–E1060, 1999.—The purpose of this study was to determine if the improvement in insulin sensitivity with exercise training is associated with enhanced phosphatidylinositol 3-kinase (PI 3-kinase) activity. Nine sedentary men were studied before and after 7 days of exercise training (1 h/day, 75% maximal oxygen consumption). Insulin sensitivity was determined with a euglycemic-hyperinsulinemic glucose clamp in the sedentary state and 15–17 h after the final exercise bout. PI 3-kinase activity was determined from samples (vastus lateralis) obtained in the fasted condition and after 60 min of submaximal insulin stimulation during the clamp. After exercise, glucose infusion rate increased (P < 0.05) significantly (means ± SE, 7.8 ± 0.5 vs. 9.8 ± 0.8 mg·kg⁻¹·min⁻¹), indicating improved insulin sensitivity. Insulin-stimulated (insulin stimulated/fasting) phosphotyrosine immunoprecipitable PI 3-kinase activity also increased significantly (P < 0.05) with exercise (3.1 ± 0.8-fold) compared with the sedentary condition (1.3 ± 0.1-fold). There was no change in fasting PI 3-kinase activity. These data suggest that an enhancement of insulin signal transduction in skeletal muscle may contribute to the improvement in insulin action with exercise.

insulin signaling; contractile activity; striated muscle; physical activity, phosphatidylinositol 3-kinase

RECENT FINDINGS have increased our understanding of the cellular events leading to insulin-stimulated glucose transport (for reviews see Refs. 7, 18, 30). Briefly, the process is initiated with ligand binding to the insulin receptor (IR). The resultant autophosphorylation triggers IR tyrosine kinase activity leading to phosphorylation and activation of a family of IR substrates (IRS). Insulin also stimulates the recruitment of phosphatidylinositol 3-kinase (PI 3-kinase) from the cytosol to a low-density fraction adjacent to the IRS protein (7, 18, 30, 32). The subsequent docking of PI 3-kinase to the insulin receptor and IRS via Src homology-2 domains increases the catalytic activity of PI 3-kinase, resulting in phosphorylated lipid products (32). It is currently believed that these lipids then activate undefined downstream signaling pathways, one or more of which result in the translocation of glucose transporters (GLUT-4) to surface cell membrane and facilitation of sugar transport into the cell (7, 18, 30, 32).

PI 3-kinase is an important regulatory step controlling glucose transport (7, 32, 33). Studies with a variety of biochemical techniques and pharmaceutical agents have demonstrated that PI 3-kinase activation is necessary for insulin-mediated glucose transport to occur in insulin-sensitive tissues such as skeletal muscle (31, 32, 33). Thus, although the signaling components downstream of PI 3-kinase are not characterized, a specific role for PI 3-kinase in insulin-stimulated glucose transport is apparent (32, 33).

Exercise training is known to enhance insulin sensitivity (12). The relationship between PI 3-kinase activity and the improvement in insulin action with exercise, however, is not clear. Studies examining insulin-stimulated PI 3-kinase activity after a single exercise bout have shown an increase (35), or conversely, a reduction (10, 34), in activity. Studies examining the effect of chronic training (9 wk) have documented an increase in the mRNA of PI 3-kinase and IRS-1 in rodent skeletal muscle, but the insulin-stimulated activity of the proteins was not measured (21, 22). Studies in human skeletal muscle are also lacking. Therefore, the purpose of the current study was to determine if insulin-stimulated PI 3-kinase activity is altered in human skeletal muscle with exercise training. A 7-day training model was selected because we and others have previously documented enhanced insulin sensitivity in populations ranging from young subjects to insulin-resistant individuals without a change in body composition (5, 6, 14, 19, 28). Improvements in insulin action can thus be primarily attributed to the effects of increased contractile activity (5, 6, 14, 19, 28).

METHODS

Experimental design. Healthy, young but sedentary men were studied before and after 7 consecutive days of exercise training (1 h/day, 75% maximal oxygen consumption [V̇O₂max], on a cycle ergometer). V̇O₂max and body composition were initially measured to verify that subjects were within normal sedentary values (23, 27) and to determine training intensity. Insulin sensitivity was determined with a 2-h hyperinsulinemic-euglycemic clamp in the sedentary condition and 15–17 h after the final training bout. A muscle sample was obtained from the vastus lateralis in the fasted condition and after 60 min of insulin infusion during the clamp for subsequent determination of fasting and insulin-stimulated PI 3-kinase activity. Insulin action (glucose infusion rate) and PI 3-kinase activity were compared before and after the 7 days of training.

Subjects. Subjects consisted of nine sedentary, young men that had not engaged in a regular exercise program for at
least the previous 12 mo. Subject characteristics were (means ± SE) age, 21.8 ± 0.9 yr; height, 176.3 ± 0.9 cm; mass, 78.9 ± 3.7 kg; body fat percentage, 15.5 ± 2.2%; and body mass index, 25.3 ± 1.2 kg/m². Subjects provided written informed consent before participation.

Hyperinsulinemic-euglycemic clamp. A hyperinsulinemic-euglycemic clamp was performed according to modification of the method of DeFronzo et al. (8) to determine insulin sensitivity. Subjects reported for the clamp at 0600 after a 12-h overnight fast. An intravenous catheter was placed in an antecubital vein for infusion of insulin and glucose. Another catheter was placed retrograde in a dorsal hand vein for blood sampling. This hand was kept in a warming box at 60°C for the collection of arterialized blood. Four samples were obtained at 10-min increments to determine fasting glucose and insulin concentration. A primed continuous infusion of insulin (Humulin, Eli Lilly, Indianapolis, IN) at a submaximal dosage of 100 µU·m⁻²·min⁻¹ was then initiated; 4 min after the start of insulin infusion, a variable 20% glucose infusion was begun. Blood samples were obtained every 5 min, centrifuged, and autoanalyzed for serum glucose (Beckman glucose II analyzer, Fullerton, CA). Adjustments in glucose infusion rate were made as needed to maintain euglycemia. Samples for insulin were obtained every 10 min and centrifuged, and plasma was stored at −70°C for subsequent analysis. Insulin concentration was determined by microparticle enzyme immunoassay (IMx, Abbott Labs, Abbott Park, IL). Glucose infusion rate was calculated from the final 30 min of the clamp.

Muscle PI 3-kinase activity. A muscle sample was obtained from the vastus lateralis with the percutaneous needle muscle biopsy technique (16). One biopsy was obtained in the fasted state before initiation of the glucose clamp. A second biopsy was obtained 60 min after initiation of insulin infusion. We have previously reported that insulin stimulation of PI 3-kinase is maximal at 60 min of a 100 µU·m⁻²·min⁻¹ glucose clamp (15). The fasting samples (pre- and posttraining) were obtained from the same leg, and the insulin-stimulated samples (pre- and posttraining) were obtained from the contralateral leg, as in a previous study (15). Muscle samples were immediately frozen in liquid nitrogen for subsequent analysis of PI 3-kinase activity.

PI 3-kinase was assayed according to the procedure of Goodyear et al. (10) with minor modifications (15). Muscle was homogenized in (1:10, wt/vol) ice-cold homogenization buffer containing 1% Nonidet P-40 in a ground-glass mortar and pestle for 30 s. The homogenate was subsequently solubilized for 60 min at 4°C on a rotator and then centrifuged at 30,000 g for 1 h. Fifty microliters of supernatant were withdrawn for protein concentration (bicinchoninic protein assay, Pierce Products, Rockford, IL) determinations. Equal volumes of supernatant (750 µl) were incubated overnight on a rotator at 4°C with antiphosphotyrosine antibody conjugated to agarose beads (Sigma-A806). The immunoprecipitate was pelleted at 4°C for 10 s at 13,000 g and washed three times with 1% Nonidet P-40 in phosphate-buffered saline (pH 7.4) containing 100 mM Na₂VO₄, three times in ice-cold 100 mM Tris, pH 7.5, with 100 mM LiCl and 100 mM Na₂VO₄, and two times with ice-cold 10 mM Tris (pH 7.5) containing 100 mM NaCl, 1 mM EDTA, and 100 mM Na₂VO₄. The pellet was resuspended in 50 µl ice-cold 10 mM Tris (pH 7.5) containing 100 mM NaCl, 1 mM EDTA, 10 µl of 100 mM MgCl₂, and 10 µl phosphatidylinositol (2 µg/µl sonicated for 60 s in 10 mM Tris, pH 7.5, with 1 mM EDTA). The reaction was started by the addition of 10 µl of 440 µM ATP containing 30 µC [³²P]ATP. After 10 min, the reaction was stopped with the addition of 20 µl of 8 N HCl, and labeled lipid was extracted with 160 µl methanol-chloroform (1:1, vol/vol). After centrifugation for 60 s at 13,000 g, the lower phase (50 µl) was separated by thin-layer chromatography on silica gel plates coated with 1% (wt/vol) potassium oxalate in a mobile phase of chloroform-methanol-water-ammonium hydroxide (60:47:11:3.2, vol/vol/vol/vol). The reaction product was visualized on a phosphorimager and quantified by densitometry (ImageQuant, LaJolla, CA). The pre- and posttraining samples for each subject were run on the same plate. Data are expressed as arbitrary units normalized to protein content of the individual samples.

Cardiovascular fitness and body composition. VO₂max was measured during incremental exercise on an electrically braked cycle ergometer (Lode, Diversified, Brea, CA). Oxygen consumption was measured with open-circuit spirometry with a metabolic cart (model 2900, Sensor Medics, Anaheim, CA). A 12-lead electrocardiogram (EKG) recorded heart rate and EKG tracings. The maximal exercise test was used 1) to determine if the subject was sedentary as classified by VO₂max (27) and 2) to determine the heart rate and oxygen consumption needed to elicit the desired intensity (~75% VO₂max) during the 7 days of training. Body composition was determined by body fat percentage measured with seven-site skinfolds (17) and body mass index. Only subjects within normative values for their age were studied (23, 27).

Exercise training. Subjects exercised 1 h/day for 7 consecutive days on a cycle ergometer. Workload was adjusted to achieve ~75% of VO₂max as determined from Douglas bags collected at minute 5 and every subsequent 15 min of exercise. Heart rate was monitored with telemetry (Polar, Stamford, CT) to also gauge exercise intensity. All subjects exercised continuously for the 1 h during the 7 days of training. A rating of perceived exertion (3) was obtained every 15 min. Body weight was recorded before and after the 7 days of training.

Statistics. Means and standard errors were calculated for the descriptive data of the subjects (body composition and VO₂max). Body mass, glucose infusion rate, and plasma glucose and insulin concentrations from the clamp were compared before and after training with repeated-measures ANOVA. The increase in PI 3-kinase activity with insulin stimulation (insulin stimulated/fasting) before and after training was also compared with ANOVA. Data are presented as means ± SE. Statistical significance was accepted as P < 0.05.

RESULTS

Subjects. Body mass did not change with the 7 days of exercise training (78.9 ± 3.7 vs. 79.5 ± 3.5 kg). The exercise characteristics of the subjects are presented in Table 1. Rating of perceived exertion (3) during training averaged 14.0 ± 0.3, which corresponded to a perception of somewhat hard to hard. Mean heart rate during the exercise bouts was 155.3 ± 1.0 beats/min.

Insulin action. Fasting plasma glucose (85.4 ± 2.3 vs. 86.8 ± 1.9 mg/dl) and insulin (2.7 ± 0.9 vs. 3.9 ± 1.4 µU·ml⁻¹) did not change during the 7 days of training.

Table 1. Exercise characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Means ± SE</th>
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<tbody>
<tr>
<td>VO₂ max, l/min</td>
<td>3.35 ± 0.17</td>
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<tr>
<td>Maximal heart rate, beats/min</td>
<td>196.0 ± 1.4</td>
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<tr>
<td>Maximal cycle power, watts</td>
<td>216.7 ± 5.9</td>
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<tr>
<td>VO₂ during training, l/min</td>
<td>2.63 ± 0.11</td>
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<tr>
<td>%VO₂ max</td>
<td>79.2 ± 2.0</td>
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Values are means ± SE; n = 9. VO₂, oxygen consumption; VO₂ max, maximal VO₂.
µU/ml) did not change with the 7 days of exercise training. There were no significant differences in plasma glucose or insulin concentrations for the sedentary vs. exercise conditions at any time points during the clamp. Plasma insulin and glucose concentrations during the clamp are presented in Fig. 1.

Glucose infusion rate (GIR) during the final 30 min of the clamp increased significantly \((P < 0.05)\) with the 7 days of training, as presented in Fig. 2. GIR increased from \(7.8 \pm 0.5\) to \(9.8 \pm 0.8\) mg·kg\(^{-1}\)·min\(^{-1}\) with exercise, indicating an improvement in insulin sensitivity. The mean increase in GIR with training was \(1.3 \pm 0.1\)-fold. This increase is within the range (1.2 to 1.4-fold) of other exercise training studies (9, 25).

PI 3-kinase activity. The change (postexercise/preexercise) in fasting PI 3-kinase activity after the 7 days of training was minimal \((0.93 \pm 0.2\)-fold\). This is in agreement with other data where a single exercise bout did not alter fasting PI 3-kinase activity in either rodent or human skeletal muscle (10, 34, 35). To obtain the increase in PI 3-kinase with insulin stimulation, PI 3-kinase activity at 60 min of the clamp was divided by the respective fasting value for each subject before and after training. The increase in insulin-stimulated PI 3-kinase activity before and after training is presented in Fig. 3. In the sedentary condition, insulin increased PI 3-kinase activity in human skeletal muscle by \(1.3 \pm 0.1\)-fold. This is lower than we previously reported \((\sim 8\)-fold, Ref. 15). The subjects in our previous study (15), however, were leaner \((13.1 \pm 1.4\%)\) and physically active, which may explain the difference between results. Direct comparison with other in vivo human work (2) is difficult because a different muscle sampling time (40 min) and insulin infusion rate (17.6 nmol·kg\(^{-1}\)·min\(^{-1}\)) were utilized; the current data, however, approximate the range reported by others (0- to 2-fold) for in vivo insulin-stimulated tyrosine phosphorylated PI 3-kinase activity in sedentary human skeletal muscle with a submaximal insulin infusion (2). After the 7 days of exercise, insulin increased PI 3-kinase activity by \(3.1 \pm 0.8\)-fold (Fig. 3). The increase in PI 3-kinase activity with insulin was significantly greater \((P < 0.05)\) after the 7 days of training vs. the sedentary condition.

**DISCUSSION**

Although exercise improves insulin-mediated glucose transport, the cellular mechanism(s) responsible remains to be defined (12). The main finding of the present study was that a relatively short period of exercise training (7 days) augmented insulin-stimulated PI 3-kinase activity, a key step in the signaling cascade leading to glucose transport (32). This finding suggests that an enhancement in insulin signal transduction may, at least in part, contribute to the increment in insulin action and glucose transport evident with exercise and/or exercise training in human skeletal muscle. It should be emphasized that the training regimen did not produce weight loss. Any effect on PI 3-kinase activation was thus primarily a result of
contractile activity rather than changes in body composition.

There is relatively little information in human skeletal muscle concerning the impact of exercise training on insulin sensitivity. Repeated exercise bouts do not appear to enhance insulin binding and insulin receptor tyrosine kinase activity (see Ref. 12). In support of postreceptor adaptations, Kim et al. (21, 22) reported that 9 wk of exercise training increased the mRNA of IRS-1 and PI 3-kinase in rodent skeletal muscle. Although the training stimulus in the current study was relatively acute, GLUT-4 protein content has been reported to increase in human skeletal muscle after 5–7 days of similar training (6, 13, 16, 26). This observation suggests that components of the glucose transport pathway can rapidly adapt with exercise training.

There are potentially conflicting findings concerning the impact of muscle contraction on PI 3-kinase activity. Goodyear et al. (10) reported that prior electrically evoked muscle contraction in rodents paradoxically blunted insulin-stimulated IRS-1 and IR-associated PI 3-kinase activity. Similar results were obtained by Wojtaszewski et al. (34) in human skeletal muscle because insulin-stimulated, IRS-1-associated PI 3-kinase activity was reduced 3 h after a single exercise bout (60 min). Both of these authors (10, 34) concluded that with exercise, PI 3-kinase activity and improved insulin-stimulated glucose uptake may not always be linked.

The discrepancy between findings may be explained by the pool of PI 3-kinase studied. In experiments where insulin-stimulated PI 3-kinase activity was augmented with exercise (Fig. 3; Ref. 35), enzyme activity was determined after immunoprecipitation with an antiphosphotyrosine antibody. Antiphosphotyrosine immunoprecipitates at least 95% of the insulin-stimulated PI 3-kinase pool (24). In contrast, studies demonstrating a reduced exercise effect on insulin-stimulated PI 3-kinase activity used antibodies specific to either the insulin receptor or IRS-1 (10, 34). One interpretation of these findings (10, 34, 35) is that exercise may increase insulin-stimulated PI 3-kinase activity by recruiting a pool not associated with either the insulin receptor or IRS-1. In agreement, Zhou and Dohm (35) reported that insulin-induced IRS-1 tyrosine phosphorylation did not change after a single exercise bout despite an increase in insulin-stimulated PI 3-kinase activity. This prompted the authors to hypothesize that exercise enhances the recruitment of PI 3-kinase to IRS-2 (35). In support of a redundant and compensatory pattern, insulin can still stimulate glucose uptake and PI 3-kinase activity, albeit to a lesser degree in IRS-1 knockout mice (1). In adipocytes from obese individuals, IRS-2 is the major docking protein in the face of markedly reduced IRS-1 levels (29).

The concept of different pools is indirectly supported by the existence of seven PI 3-kinase isoforms that are regulated differently by insulin under normal conditions and in the insulin-resistant state (20, 31). Exercise training may preferentially activate and/or increase the concentration of PI 3-kinase isoforms that stimulate glucose transport. The functional differences among these isoforms are still, however, unclear (20, 31, 32). A final consideration is that PI 3-kinase activity may be potentiated with exercise by a factor outside of the insulin-signaling cascade. The minimal data available (10, 21, 22, 34, 35), however, only permit conjecture concerning the cellular mechanism responsible for the increase in PI 3-kinase activity reported here (Fig. 3). Regardless, the present findings provide evidence that exercise does enhance insulin signal transduction at the level of PI 3-kinase. This effect was evident in human skeletal muscle with a relatively acute training stimulus.

The data of the current study are potentially relevant to some insulin-resistant conditions. Insulin-stimulated PI 3-kinase activity in IRS-1, IRS-2, and phosphotyrosine immunoprecipitates is markedly reduced in the skeletal muscle of aged, insulin-resistant rodents (4). Insulin-stimulated PI 3-kinase activity from both IRS-1 and phosphotyrosine immunoprecipitates is also dramatically decreased in the skeletal muscle of indi-
individuals with type II diabetes and obesity (2, 11). The present findings thus provide a potential mechanistic explanation for observations where a virtually identical 7-day exercise training protocol improved insulin action in aged, obese, and type II diabetic individuals (5, 6, 14, 19, 28).

In conclusion, 7 days of exercise training (60 min/day, ~75% VO_{2\text{max}}) increased insulin sensitivity in healthy, young, but sedentary men. The improvement in insulin action was accompanied by increased PI 3-kinase activity in aged, obese, and type II diabetic individuals (5, 6, 14, 19, 28).

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