Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in middle-aged men

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EXERCISE TRAINING improves insulin action in middle- to older-aged individuals (7, 8, 18, 33). This effect occurs relatively rapidly, with enhanced insulin action evident after as little as seven consecutive days of physical activity [50–60 min/day at 50–75% peak oxygen consumption (V'O_2 peak)] (7, 8, 33). The ability to improve insulin action with exercise in an older population is important, because the insulin resistance of aging begins early in middle age (30–40 yr) and is associated with an enhanced risk for coronary artery disease, obesity, and type 2 diabetes (11, 13, 20, 31). Although exercise improves insulin action in middle- to older-aged subjects, the cellular mechanisms involved are not yet evident. Recent findings have increased our understanding of the cellular events leading to insulin-stimulated glucose transport (for reviews see Refs. 10, 22, 34). Briefly, the process is initiated with ligand binding to the insulin receptor and tyrosine kinase activation that leads to phosphorylation and activation of a family of insulin receptor substrates (IRS). The subsequent docking of phosphatidylinositol (PI) 3-kinase to an IRS results in phosphorylated lipid products, which activate yet-undefined downstream signaling pathways (36). This signaling process ultimately induces translocation of the insulin-sensitive glucose transporter (GLUT-4) to skeletal muscle membranes and facilitates sugar transport into the cell (10, 21, 34, 36). After 7 days of exercise training, GLUT-4 and insulin action increased by the same relative magnitude in young and middle-aged subjects (8), indicating that older individuals retain the ability to respond to the exercise stimulus.

The effect of exercise training on the signaling events that induce GLUT-4 translocation are not, however, evident, particularly in aged skeletal muscle (16). Several studies have focused on PI 3-kinase, as this protein is directly involved in insulin-mediated glucose transport (10, 35, 36, 37). In young subjects, exercise training increased insulin-stimulated PI 3-kinase activity in conjunction with insulin action (19, 26). However, Cusi et al. (9) reported no improvement in insulin-stimulated PI 3-kinase activity in obese individuals and type 2 diabetics after a single exercise bout; insulin action was also not enhanced. This finding (9) suggests that the insulin-signaling pathway may also be resistant to the exercise training stimulus (i.e., consecutive

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days of exercise as opposed to a single exercise bout; Ref. 9) at the level of PI 3-kinase in insulin-resistant individuals. The effect of exercise training that improves insulin action on PI 3-kinase has not, however, been studied in an older insulin-resistant population. The purpose of the current study was, therefore, to examine the effect of a short-term exercise training protocol demonstrated to improve insulin action (8) on insulin-mediated PI 3-kinase activity in middle-aged subjects. Due to our previous finding of enhanced PI 3-kinase in young subjects with physical activity (19), our hypothesis was that insulin-stimulated PI 3-kinase activity in skeletal muscle from middle-aged individuals would increase with exercise training, in conjunction with improved insulin action.

**METHODS**

**Study design.** Subjects were assessed for suitability by an interview, health history questionnaire, and medical clearance. Preliminary assessments included body composition (and a \( \text{V}_{\text{O}_2\text{peak}} \) test) to ensure that subjects were sedentary, 2) to ensure that there was no overt evidence of heart disease, and 3) to calculate the workload used during exercise training. After these preliminary measurements, a pretraining hyperinsulinemic euglycemic clamp was administered with muscle biopsies performed before and 60 min into the clamp. A period of 7 consecutive days of exercise training (cycle ergometer) was initiated within 14 days of the clamp. Fifteen to seventeen hours after the last training bout, a posttraining hyperinsulinemic euglycemic clamp was administered, and muscle biopsies were performed before and 60 min into the clamp. PI 3-kinase activity was determined in each of the muscle samples, as this was the primary focus of the study. There was sufficient material remaining in four subjects to determine protein kinase B (Akt) activity.

**Subjects.** All subjects read and provided informed consent to acknowledge the risks and procedures involved before participating in this study. Approval by the East Carolina University Policy and Review Committee on Human Research was obtained before any testing was performed. Nine untrained, middle-aged (50–70 yr) Caucasian men were recruited as volunteers. Subjects exhibited no evidence of cardiovascular disease, were not taking medications that could affect carbohydrate metabolism or exercise tolerance, and did not have orthopedic difficulties that could hinder exercising. Subjects had not performed regular exercise for at least 2 yr before initiation of the study.

\( \text{V}_{\text{O}_2\text{peak}} \) was determined using a Lode electronically braked cycle ergometer (Diversified, Brea, CA) during an incremental exercise test to voluntary exhaustion. Each subject was monitored with a 12-lead electrocardiogram in the final minute of every 3-min stage. Subjects were asked to exercise to exhaustion and until at least two of the following events.

**Exercise training.** All exercise training was performed in the laboratory under the supervision of an exercise physiologist (C. J. Tanner). Training duration was 60 min/session; frequency was 1 session/day for 7 consecutive days. Exercise intensity was maintained at 70% of the subject’s \( \text{V}_{\text{O}_2\text{peak}} \) by analysis of expired gases every 5–15 min. Heart rate was continuously recorded using a Polar heart rate monitor (Polar USA, Stamford, CT) to ensure an adequate training intensity. This exercise program has been used previously in this laboratory (8, 19) and by others (7, 33) to improve insulin action without weight loss in insulin-resistant individuals.

**Muscle biopsy.** Muscle samples of ~60 mg were obtained from the lateral portion of the vastus lateralis, before training and 15–17 h after the last training bout for the determination of PI 3-kinase and Akt activity. Before initiation of the clamp, a biopsy was obtained from the left leg as the basal, fasting sample. The second biopsy was obtained from the right leg after 60 min of insulin exposure. The same sequence was performed after the 7 days of training. Tissue was obtained 60 min into the clamp, as we have previously demonstrated optimal PI 3-kinase activation at this time point with the insulin dosage and clamp procedure used in the present study (17). Other studies (2, 40, 41) have also obtained muscle biopsies at similar time points (40–100 min) during a glucose clamp when measuring insulin-signaling events.

**PI 3-kinase assay.** PI 3-kinase activity was assayed after immunoprecipitation with an anti-phosphotyrosine antibody according to methods previously used in this (17, 19) and other (14, 15) laboratories. Skeletal muscle from the biopsies was homogenized in ice-cold buffer (1:10 wt/vol) containing 1% (vol) Nonidet P-40 for 30 s. The supernatant was incubated overnight at 4°C with anti-phosphotyrosine conjugated to agarose beads (Sigma A-1806). Labeled lipids were extracted with 300 μl of methanol-chloroform (1:1, vol/vol), reaction products were visualized on a phosphorimager, and radioactivity was quantified by densitometry. Results were normalized to protein content.

**Akt assay.** Akt activity was determined as previously described (23, 24). Twenty milligrams of muscle were homogenized using a polytron at one-half maximum speed for 1 min on ice in 500 μl of buffer A (in mM: 20 Tris, pH 7.5, 5 EDTA,
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10 Na₄P₂O₇, 100 NaF, 2 Na₃VO₄) containing 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, and 10 mg/ml leupeptin. Tissue lysates were solubilized by continuous stirring for 1 h at 4°C and centrifuged for 10 min at 14,000 g. Muscle lysates (200 mg protein) were subjected to immunoprecipitation for 4 h at 4°C with 3 mg of Akt antibody, which recognizes both Akt1 and Akt2 (Upstate Biotechnology, Lake Placid, NY) coupled to protein G-Sepharose beads (Pharmacia Biotech, Piscataway, NJ). Immune pellets were washed, and Akt activity was determined as previously described (24, 25).

Statistical analysis. PI 3-kinase activity was expressed as degree of stimulation over fasting by dividing the measured activity after 60 min of insulin exposure by the fasting activity. Repeated-measures analysis of variance (ANOVA) on log-normalized and non-log-normalized data was used to test for a difference between insulin stimulation before and after short-term exercise training. All other variables were compared with repeated-measures ANOVA. Statistical significance was denoted at the \( P < 0.05 \) level, and data are presented as means ± SE.

RESULTS

Subjects. Physical characteristics of the subjects are presented in Table 1. The exercise characteristics of the subjects are presented in Table 2. Subjects exercised at \(-70\%\) of \( \dot{V}_{O_2}\text{peak} \) during the training sessions. Total body weight, which was measured each day immediately before exercise, was not significantly altered during the 7 days of training \((P = 0.49)\).

Insulin action. Fasting plasma insulin concentration tended to decrease with the 7 days of training \((8.8 ± 1.4 \text{ vs. } 6.8 ± 1.4 \mu\text{g/ml for pre- vs. postraining, respectively, } P = 0.10)\). Fasting plasma glucose concentration also tended to decrease with physical activity \((100.4 ± 5.1 \text{ vs. } 97.0 ± 4.9 \text{ mg/dl, } P = 0.09)\). There were no significant differences \((P = 0.38)\) in insulin or glucose concentration during the final 30 min of the clamp for before vs. after short-term exercise training (Fig. 1).

M-values were used as the index of insulin action (11). Mean M-value before training was 6.6 ± 0.6 vs. 8.7 ± 0.8 mg·kg⁻¹·min⁻¹ after training. This represented a 33% mean improvement in insulin action \((P < 0.001, \text{ Fig. 2})\).

PI 3-kinase activity. Insulin-stimulated PI 3-kinase activity was expressed as mean degree of stimulation over fasting activity and is depicted before and after the 7 days of exercise training in Fig. 3. Mean insulin-stimulated PI 3-kinase activity did not change \((1.8 ± 0.8 \text{ vs. } 1.8 ± 0.7 \text{ mean degree of change over fasting for pre- vs. postraining, respectively; } P = 0.63)\) with short-term training (Fig. 3). These degrees of increase in PI 3-kinase activity with insulin were within the ranges reported in other studies examining the skeletal muscle of insulin-resistant humans during a glucose clamp \([\text{range } 0–1.5 \text{ degree of stimulation over fasting with insulin exposure (2, 9, 24, 28)}]\). Before the 7 days of exercise training, insulin-stimulated anti-phosphotyrosine-associated PI 3-kinase activity was not statistically different \((P = 0.93)\) from fasting PI 3-kinase activity \((45.0 ± 16.0 \text{ vs. } 67.9 ± 19.9 \text{ arbitrary units for fasting vs. insulin-stimulated activity, respectively})\). After training, insulin again did not significantly increase \((P = 0.96)\) anti-phosphotyrosine-associated PI-3 kinase activity over fasting activity \((46.7 ± 13.9 \text{ vs. } 67.1 ± 21.0 \text{ arbitrary units for fasting vs. insulin-stimulated activity, respectively})\). These data are in agreement with findings obtained in other in vivo studies of insulin-resistant subjects (2, 9, 24), where insulin did not significantly increase PI-3 kinase activity over fasting activity in skeletal muscle.

Akt activity. As presented in Fig. 4, insulin-stimulated Akt activity did not change with exercise training. Insulin infusion during the clamp enhanced Akt activity a mean of 1.3 ± 0.1-fold before exercise training and a mean of 1.4 ± 0.2-fold after training; these values were not significantly different \((P = 0.61)\). To our knowledge, there are no insulin-stimulated Akt data in insulin-resistant human muscle for the purpose of comparison. Insulin did not increase Akt activity compared with fasting before training \((2,357 ± 74 \text{ vs. } 2,573 ± 112 \text{ arbitrary units for fasting vs. insulin-stimulated, respectively; } P = 0.18)\). However, after training, insulin exposure did increase Akt activity compared with fasting \((2,145 ± 158 \text{ vs. } 2,704 ± 82 \text{ arbitrary units for fasting vs. insulin-stimulated, respectively; } P = 0.04)\).

DISCUSSION

PI 3-kinase is an important regulatory step of insulin signal transduction in insulin-sensitive cells (36, 37). Studies utilizing various biochemical techniques and pharmaceutical agents have demonstrated that PI 3-kinase is a necessary component of insulin-mediated glucose transport (35–37). Thus, although the signaling components downstream of PI 3-kinase are not well characterized, a specific role for PI 3-kinase in insulin-stimulated glucose transport is apparent (35–37).

In previous work from our laboratory (19), an identical 7-day exercise training program increased insulin-stimulated PI-3 kinase activity about threefold.

Table 1. Selected subject characteristics \((n = 9)\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>58.1 ± 1.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.0 ± 1.8</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>93.4 ± 5.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.9 ± 1.4</td>
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<tr>
<td>Body fat, %</td>
<td>24.8 ± 1.9</td>
</tr>
</tbody>
</table>

Table 2. Characteristics during maximal exercise and 7 days of exercise training \((n = 9)\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal heart rate, beats/min</td>
<td>164.2 ± 5.1</td>
</tr>
<tr>
<td>Training heart rate, beats/min</td>
<td>132.0 ± 4.2</td>
</tr>
<tr>
<td>Training heart rate, %max</td>
<td>81.4 ± 2.2</td>
</tr>
<tr>
<td>( \dot{V}_{O_2}\text{peak}, l/min}</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Training ( V_{O_2}), l/min</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Training ( V_{O_2}), %peak</td>
<td>70.7 ± 1.9</td>
</tr>
</tbody>
</table>
compared with the nonexercising, sedentary condition in the skeletal muscle of young individuals (25 yr) in concordance with improved insulin action. In a cross-sectional design, Kirwan et al. (26) recently reported that insulin-stimulated IRS-1-associated PI 3-kinase activity was at least doubled in the skeletal muscle of young (mean age of 24 yr), endurance-trained subjects compared with their sedentary counterparts. In rodents, enhanced PI 3-kinase activity with insulin stimulation was reported after 1 and 5 days of exercise training (6). In contrast to these data, the main finding of the present study was that insulin-stimulated PI 3-kinase activity did not increase with short-term exercise training in the skeletal muscle of middle-aged men (Fig. 3). This result suggests that the insulin-signaling cascade, at least at the level of PI 3-kinase,

Fig. 1. Plasma glucose (triangles) and insulin (squares) concentrations during the euglycemic hyperinsulinemic glucose clamp before (open symbols) and after (closed symbols) 7 days of exercise training (n = 9).

Fig. 2. Insulin action (M-value) before and after 7 days of training at ~70% peak O2 consumption in middle-aged (58 yr) men (▲, n = 9). *Significantly different (P < 0.0001) between pre- and posttraining.

Fig. 3. Mean insulin-stimulated phosphatidylinositol (PI) 3-kinase activity (insulin stimulated/fasting activity) before and after 7 days of exercise training in middle-aged men (n = 9).
may respond differently to the exercise training stimulus, depending upon the population examined.

The differing response of insulin-stimulated PI 3-kinase activity to exercise training in young (19, 26) vs. older individuals (Fig. 3) may involve obesity status and the initial presence of insulin resistance. It has been reported that aging (5), obesity (9, 15, 25), and type 2 diabetes (2, 9, 24) dramatically decrease the responsiveness of PI 3-kinase to insulin. For example, Carvalho et al. (5) reported an ~90% decline in insulin-stimulated PI 3-kinase activity in skeletal muscle from young (2 mo) vs. aged (20 mo) male Wistar rats. In type 2 diabetic and obese subjects, insulin-stimulated anti-phosphotyrosine-associated PI 3-kinase activity in skeletal muscle is virtually abolished (2, 9), as was the case in the present study (RESULTS, Fig. 3). A marked inability to activate PI 3-kinase with insulin is thus displayed in conditions typically associated with insulin resistance.

In an attempt to overcome this resistance in activation of PI 3-kinase, Cusi et al. (9) subjected obese subjects or individuals with type 2 diabetes to a single, relatively mild (60 min, 65% VO2 max) exercise bout and studied components of the insulin-signaling pathway 24 h after the exercise session. In these insulin-resistant individuals, a single exercise bout did not increase either insulin-stimulated IRS-1 or phosphotyrosine-associated PI 3-kinase activity, nor did it enhance insulin action compared with preexercise values (9). Phosphorylation of the insulin receptor and IRS-1 along with glycogen synthase activity did, however, increase. These authors (9) concluded that a single bout of exercise could not overcome the defect in the insulin-signaling pathway present with insulin resistance and that PI 3-kinase defines a key step in the insulin-resistant state. The current data provide the important additional information that insulin signal transduction at the level of PI 3-kinase is not altered with the more robust intervention of short-term exercise training that improves insulin action in insulin-resistant sub-

jects (Figs. 2 and 3). This novel finding suggests that adaptations downstream of PI 3-kinase or alternate insulin-signaling pathways may be responsible for the improvement in insulin action with physical activity in middle-aged, insulin-resistant subjects.

To examine other potential mechanisms, we measured the activity of an additional insulin-signaling element, Akt. Akt is hypothesized to be the next step distal to PI 3-kinase in the insulin-signaling process for glucose transport (4, 27). Insulin-stimulated Akt activity can also be reduced in the insulin-resistant state (24, 28). In agreement with our PI 3-kinase data, we observed no increase in the activation of Akt with insulin after training (Fig. 4). It must be considered, however, that the Akt data were obtained in a small subset of subjects and that exercise may have different effects on the individual isoforms of Akt.

Because the magnitude of the insulin signal did not appear to change, another factor may be responsible for the improvement in insulin action with training (Fig. 2). Systemic factors such as increased muscle blood flow with training may enhance insulin-mediated glucose uptake (1). In relation to skeletal muscle, the overexpression of GLUT-4 in transgenic mice produces an approximately twofold increase in protein concentration, which is similar in scope to the exercise training response (8, 16, 18, 29, 32, 38, 39). In these transgenic animals, insulin sensitivity is enhanced. It was suggested (3) that, in obese Zucker rats, the increase in GLUT-4 with training alone compensated for the defect in insulin-stimulated GLUT-4 translocation. These data suggest that the presence of additional insulin-sensitive glucose transporters in skeletal muscle with exercise training may be sufficient to enhance whole body insulin action without an accompanying amplification of the insulin-signaling process. In support of this hypothesis, in previous work from our laboratory (8), GLUT-4 concentration increased approximately twofold in conjunction with enhanced insulin action after an identical 7-day exercise training program in similar middle-aged men. This finding (8) suggests that GLUT-4 increased to an extent similar to that in the current study, although we did not measure the concentration of this protein again.

It must be acknowledged that some aspect(s) of the insulin-signaling cascade may also have been overlooked with the current experimental design. For example, a yet-unidentified signaling step distal to PI 3-kinase or Akt could be enhanced with exercise, as well as an alternative insulin-signaling system. Also, in the present study, total PI 3-kinase activity was determined after immunoprecipitation with a phosphotyrosine antibody. This approach was used because it measures ~95% of the activated PI 3-kinase (30); we have also observed a relationship between total PI 3-kinase activity and insulin-mediated glucose uptake in humans (17). It is possible that IRS-1- or IRS-2-associated PI 3-kinase activity may have been differentially affected with training (6). There are also seven insulin-regulated PI 3-kinase isoforms, which may exhibit separate responses to training (23, 35). It must be
considered, however, that phosphotyrosine-associated PI 3-kinase activation was enhanced after 7 days of training in young individuals with the use of identical methods in our laboratory (19). It is thus logical to assume that the methodology used would have detected an increase in PI 3-kinase activity with exercise.

Exercise training improves insulin action through both acute effects from the last training bout and the chronic accumulation of successive exercise sessions (16). The current experiment was not designed to distinguish between acute and chronic influences of physical activity on insulin action. Rather, we utilized the 7-day model because it has been demonstrated to enhance insulin action in insulin-resistant subjects without a confounding change in body mass (7, 8, 33; RESULTS). An interesting finding was that exercise improved insulin action by the same relative magnitude (~30%) in both young and middle-aged subjects when we compared the current findings with previous data from our laboratory (19). This finding indicates that middle-aged individuals maintain the ability to adapt to an exercise stimulus and improve insulin action. The cellular mechanism(s) involved still, however, remains largely undefined.

In summary, a 7-day exercise training program significantly improved insulin action in insulin-resistant, middle-aged men. The improvement in insulin action with exercise was not accompanied by enhanced insulin signal transduction at the level of PI 3-kinase or Akt in skeletal muscle. These data suggest that short-term exercise training enhances insulin action in middle-aged individuals via an adaptation distal to PI 3-kinase.

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