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Regular exercise enhances insulin activation of IRS-1-associated PI3-kinase in human skeletal muscle

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Kirwan, John P., Luis F. Del Aguila, Jazmir M. Hernandez, David L. Williamson, Donal J. O'Gorman, Rebecca Lewis, and Raj K. Krishnan. Regular exercise enhances insulin activation of IRS-1-associated PI3-kinase in human skeletal muscle. *J. Appl. Physiol.* 88: 797–803, 2000.—Insulin action in skeletal muscle is enhanced by regular exercise. Whether insulin signaling in human skeletal muscle is affected by habitual exercise is not well understood. Phosphatidylinositol 3-kinase (PI3-kinase) activation is an important step in the insulin-signaling pathway and appears to regulate glucose metabolism via GLUT-4 translocation in skeletal muscle. To examine the effects of regular exercise on PI3-kinase activation, 2-h hyperinsulinemic (40 $mU \cdot m^{-2} \cdot min^{-1}$)-euglycemic (5.0 mM) clamps were performed on eight healthy exercise-trained [24 ± 1 yr, 71.8 ± 2.0 kg, maximal O_2 uptake ($\dot{V}O_{2max}$) of 56.1 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$] and eight healthy sedentary men and women (24 ± 1 yr, 64.7 ± 4.4 kg, $\dot{V}O_{2max}$ of 44.4 ± 2.7 $ml \cdot kg^{-1} \cdot min^{-1}$). A [6,6-^{2}H]glucose tracer was used to measure hepatic glucose output. A muscle biopsy was obtained from the vastus lateralis muscle at basal and at 2 h of hyperinsulinemia to measure insulin receptor substrate-1(IRS-1)-associated PI3-kinase activation. Insulin concentrations during hyperinsulinemia were similar for both groups (293 ± 22 and 311 ± 22 pM for trained and sedentary, respectively). Insulin-mediated glucose disposal rates (GDR) were greater ($P < 0.05$) in the exercise-trained compared with the sedentary control group (9.22 ± 0.95 vs. 6.36 ± 0.57 $mg \cdot kg$ fat-free $mass^{-1} \cdot min^{-1}$). Insulin-stimulated PI3-kinase activation was also greater ($P < 0.004$) in the trained compared with the sedentary group (3.8 ± 0.5- vs. 1.8 ± 0.2-fold increase from basal). Endurance capacity ($\dot{V}O_{2max}$) was positively correlated with PI3-kinase activation ($r = 0.53$, $P < 0.04$). There was no correlation between PI3-kinase and muscle morphology. However, increases in GDR were positively related to PI3-kinase activation ($r = 0.60$, $P < 0.02$). We conclude that regular exercise leads to greater insulin-stimulated IRS-1-associated PI3-kinase activation in human skeletal muscle, thus facilitating enhanced insulin-mediated glucose uptake.

glucose metabolism; exercise training; insulin action; muscle; enzymes; insulin receptor substrate-1; phosphatidylinositol 3-kinase

EXERCISE HAS LONG BEEN USED to successfully treat and prevent insulin resistance and Type 2 diabetes. Nevertheless, our knowledge of the mechanisms that explain how exercise training improves insulin-mediated glucose regulation remains unclear. A number of investigators have shown increased skeletal muscle insulin sensitivity among people who exercise on a regular basis (14, 26, 29, 34). Mechanisms that have been proposed to account for these exercise-related improvements include prereceptor events [increased blood flow (14) and muscle fiber morphology, as well as postreceptor adaptations], increased glucose transport and concentration of GLUT-4 glucose transporters in skeletal muscle (11, 17, 22), and greater activity of the enzymes hexokinase II (30) and glycogen synthase (13, 14). Recently, investigators have successfully identified postreceptor steps that include an intracellular insulin-signaling cascade that leads to cellular glucose uptake in skeletal muscle (6, 7, 31, 39, 42). To date, there has been little elucidation on the effects of exercise training on insulin signaling in human skeletal muscle at the molecular level and the contribution that such effects might have on glucose metabolism.

In skeletal muscle, insulin binds to its receptor on the plasma membrane and initiates a pleiotrophic cascade of intracellular signaling events that includes increased glucose uptake via the recruitment of a pool of intracellular glucose transporters known as GLUT-4 (19). The proximal steps in this signaling cascade involve autophosphorylation of the insulin receptor on tyrosine residues, phosphorylation of a family of sub-

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strates that includes insulin receptor substrate-1 (IRS-1) (39), and subsequent binding and activation of phosphatidylinositol 3-kinase (PI3-kinase). There is now ample evidence to demonstrate the involvement of PI3-kinase in the process of skeletal muscle glucose uptake (6, 7, 31, 42).

If the increased insulin sensitivity associated with exercise training is a postreceptor adaptation, it may be mediated by enhanced intracellular signaling leading to greater glucose uptake by the muscle. An increase in insulin-stimulated PI3-kinase activation in exercise-trained muscle could provide important information on the effects of exercise on the insulin signaling pathway and reveal new clues regarding the mechanisms responsible for the augmented insulin sensitivity that is observed among exercise-trained individuals. Therefore, the purpose of this study was to determine whether insulin-stimulated PI3-kinase activation in skeletal muscle is upregulated in men and women who exercise on a regular basis. In addition, we evaluated whether alterations in insulin-activated PI3-kinase with regular exercise are associated with enhanced insulin-mediated glucose uptake. Because muscle fiber morphology and increased perfusion of skeletal muscle have been considered as possible prereceptor adaptations that facilitate enhanced insulin action after exercise training, we also examined the relationship between insulin activation of PI3-kinase, skeletal muscle fiber composition, and capillary density in exercise-trained and sedentary subjects.

METHODS

Subjects

Eight exercise-trained men ($n = 7$) and women ($n = 1$) and eight sedentary men ($n = 6$) and women ($n = 2$) participated in the study (Table 1). All of the subjects in the trained group had engaged in aerobic exercise (running and/or cycling) 4–5 days/wk for at least 12 mo before the study. All subjects were requested to perform a "normal" workout (45–90 min, at ~80–90% maximal heart rate) no later than midafternoon on the day preceding the procedure. The study was approved by the Institutional Review Board for Human Subjects, and all volunteers signed an informed consent in accordance with Pennsylvania State University guidelines for the protection of human subjects.

Each subject performed an incremental treadmill test to determine maximal oxygen consumption ($\dot{V}O_{2max}$). Inspired air volumes were measured from pressure changes detected with a pneumotach (Hans Rudolph). Concentrations of O_2 and CO_2 were measured on an electrochemical O_2 analyzer (Applied Electrochemistry, S-3A) and infrared CO_2 analyzer (Beckman LB-2), respectively. A standard 75-g oral glucose tolerance test was performed to verify normal glucose tolerance (2). Body density was determined by hydrostatic weighing after an overnight fast according to the method of Akers and Buskirk (1). Underwater weight was determined using electronic load cells. Residual lung volume was determined during immersion by open-circuit nitrogen washout, and percent body fat was estimated using the Siri equation (37). Height was measured to the nearest 1.0 cm without shoes, and body weight was measured to the nearest 0.1 kg. To control dietary intake and physical activity, subjects ate their evening meal in the General Clinical Research Center and stayed overnight before the experimental protocol. The subjects were instructed to consume a diet that consisted of 60% of energy coming from carbohydrate, 25% from fat, and 15% from protein for the 2 days before the trial.

Table 1. *Subject characteristics*

Variable	Sedentary	Trained
Age, yr	24 ± 1	24 ± 1
Height, cm	175 ± 1	175 ± 1
Body weight, kg	64.7 ± 4.4	71.8 ± 2.0
Body mass index, kg/m^2	21.0 ± 1.2	23.4 ± 0.5
Body fat, %	14.2 ± 3.8	12.3 ± 2.0
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	44.4 ± 2.5	56.1 ± 2.5*

Values are means ± SE. There were 8 subjects per group. $\dot{V}O_{2max}$, maximal oxygen uptake. *Significant difference between groups, $P < 0.05$.

Experimental Protocol

Hyperinsulinemic-euglycemic clamps (120 min, 40 mU·m^{-2}·min^{-1} insulin and 5.0 mM glucose) were performed as originally described by DeFronzo et al. (9). After a 10- to 12-h overnight fast and ~18 h after the last exercise bout (trained only), the subjects voided morning urine and were weighed. A polyethylene catheter was inserted into an antecubital vein for infusion of insulin, glucose, [6,6-^{2}H]glucose, and potassium chloride. A second polyethylene catheter was inserted retrograde into a dorsal hand vein, and the hand was warmed in a heated box (~65°C) for sampling of arterialized venous blood (33). Hepatic glucose output was measured using a bolus (3.276 mg/kg) infusion (Harvard Apparatus, South Natick, MA) of [6,6-^{2}H]glucose (Tracer Technology, Somerville, MA) followed by a constant infusion at 0.0364 mg·kg^{-1}·min^{-1} for a 2-h baseline period and throughout the clamp. After the baseline period, a primed, continuous infusion (40 mU·m^{-2}·min^{-1}) of human insulin (Humulin, Eli Lilly, Indianapolis, IN) was initiated and maintained for a period of 2 h. Baseline blood samples were drawn before the tracer infusion and at 10-min intervals during the last 30 min of the baseline period and the last 40 min of the hyperinsulinemic clamp. Plasma glucose levels were clamped at 5.0 mM during hyperinsulinemia by use of a variable glucose infusion (20% dextrose). Blood samples for plasma glucose and insulin determination were drawn at 5- and 15-min intervals, respectively, during the clamp. Muscle biopsies were performed using the needle biopsy procedure as previously described (28). Tissue was obtained from the vastus lateralis muscle of one leg during the baseline period and from the opposite leg immediately at the 120-min time point of the clamp. Hyperinsulinemia was maintained throughout the biopsy procedure.

Analytical Procedures

Plasma glucose concentrations were measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Blood samples for insulin measurements were centrifuged at 4°C, and the plasma was stored at −70°C for subsequent analysis in duplicate by a double-antibody RIA (Linco Research, St. Charles, MO).

Blood samples for [6,6-^{2}H]glucose determination were prepared as described previously (27). The samples were centrifuged, and the plasma (200 μl) was deproteinized with 300 μl of cold acetone. After further centrifugation, the supernatant was removed and evaporated and the pentaacetate derivative of glucose was formed by addition of 100 μl of acetic anhydride-pyridine (1:1). Glucose was separated at 180°C on a 3% OV

column, and its ^2H isotopic abundance was measured by positive ion-chemical ionization mass spectrometry through the use of selective ion monitoring of mass-to-charge ratios of 333 and 331.

Muscle analysis. The muscle sample was immediately homogenized in a buffer solution (50 mM HEPES, 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM sodium pyrophosphate, 10 mM NaF, 2 mM EDTA, 2 mM $NaVO_4$, 1% NP-40, 10% glycerol, 2 μg/ml aprotinin, 5 μg/ml leupeptin, 1.5 mg/ml bentamidine, 0.2 M 4-(2-aminoethyl)benzenesulfonyl fluoride, 10 μg/ml antipain, and 0.5 μg/ml pepstatin) for subsequent analysis of IRS-1-associated PI3-kinase activation. A second sample was prepared for histochemical analysis of muscle fiber type and capillary density. The muscle sample was mounted in tragacanth gum and quickly frozen in isopentane, cooled in liquid N_2. All muscle specimens for histochemical analysis were stored at −70°C. A total of 15 serial cross sections (10 μm) of each muscle specimen were cut at −20°C in a cryostat microtome (Leica Cryocut 1800) and mounted on slides. The sections were dried at room temperature and stained for myosin ATPase (preincubation at pH 4.3 and 4.6) and with amylase-periodic acid-Schiff to visualize capillaries (3). An average of 300 muscle fibers were identified as type I and II based on the ATPase stain. Capillary counts were expressed as capillaries per fiber.

IRS-1-associated PI3-kinase activity. Protein concentration in the tissue homogenates was determined by the Bio-Rad protein assay following the manufacturer's instructions (Bio-Rad Laboratories). A 1-mg sample of total protein was immunoprecipitated with 4 μg of the IRS-1 polyclonal antibody (Upstate Biotechnology, Lake Placid, NY) and rocked overnight at 4°C. A 40-μl sample of slurry protein A-Sepharose was added to the immunoprecipitate for 2 h, and an immunocomplex was obtained by brief centrifugation at 9,000 rpm and washed three times in PBS-1% NP-40, twice in 500 mM LiCl-100 mM Tris (pH 7.6), and once in 10 mM Tris·HCl (pH 7.4), 100 mM NaCl, and 1 mM CDTA. The pellet was centrifuged one more time and washed in PI3-kinase adenosine assay buffer [20 mM Tris (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, 0.5 mM EGTA, and 120 μM adenosine]. The final pellet was resuspended in 40 μl of PI3-kinase adenosine assay buffer. A 50-μl sample of phosphatidylinositol and phosphatidylserine was dried down in a nitrogen stream and sonicated in 100 μl of 20 mM HEPES-1 mM EDTA (pH 7.4). The lipid mixture was kept on ice, and 5 μl of this mixture (2 μg/μl of phosphatidylinositol) were added to each sample. The solution was mixed by sonication and incubated for 10 min at 30°C on a heat block. A mixture consisting of 170 μCi of γ-^{32}P-labeled and 280 μM unlabeled ATP was prepared, and the reaction was started by adding 5 μl of this mixture into each sample. After 10 min at 30°C, the reaction was stopped by the addition of 200 μl of 1 N HCl to each sample. The phosphatidylinositol 3-phosphate (PI3-phosphate) was extracted with 160 μl chloroform-methanol (1:1). The phases were separated by centrifugation, and the lower organic phase was removed and separated by TLC. The radioactivity incorporated into PI3-phosphate was determined by PhosphorImaging (Molecular Dynamics, Sunnyvale, CA).

Calculations and Statistical Analysis

The rates of glucose appearance and disappearance (R_d) were calculated from plasma [6,6-^2H]glucose enrichments and the rate of tracer infusion, using the equations described by Jahoor et al. (23). When R_d was estimated as a negative value, the glucose disposal rate was assumed to be the steady-state glucose infusion rate. All values are presented as means ± SE. Differences between dependent variables were examined with two-way ANOVA. Differences between descriptive variables were examined with a one-way ANOVA. Specific mean differences were identified with a Newman-Keuls post hoc test. The α-level for statistical significance was set at 0.05.

RESULTS

Subjects were similar in age and body composition, but the trained group had a higher maximal exercise oxygen consumption compared with the sedentary group (Table 1). Muscle fiber types (51 ± 7 vs. 35 ± 7% type I, $P = 0.06$, for the trained and untrained subjects, respectively) and capillary density (2.8 ± 0.4 vs. 1.8 ± 0.1 capillaries/fiber, $P = 0.07$, for the trained and sedentary subjects, respectively) were not significantly different between the groups.

Fasting glucose and insulin levels were normal and were similar for both groups of subjects before the initiation of the glucose clamp (Table 2). Mean glucose concentrations were 5.1 ± 0.1 and 4.9 ± 0.1 mM for the trained and sedentary groups during the final 30 min (90–120 min) of the clamp (Table 2). The coefficients of variation for plasma glucose during this period were 5.6 ± 0.7% and 4.8 ± 0.5% for the trained and sedentary groups, respectively (Table 2). Insulin-mediated glucose disposal rates expressed per kilogram fat-free mass were significantly higher in the exercise-trained group compared with the untrained, sedentary group (Fig. 1).

Insulin activation of IRS-1-associated PI3-kinase was expressed as multiples of increase in activity measured in the muscle during hyperinsulinemia (120 min) with respect to the preclamp muscle sample. Activation was significantly increased in both the trained ($P < 0.0001$) and sedentary ($P < 0.003$) groups as a result of hyperinsulinemia (Fig. 2). The level of activation was significantly greater ($P < 0.004$) in the trained group compared with the sedentary control group. All of the trained subjects showed an increase in activation, seven of the untrained subjects showed an increase, and one showed no change. Correlation analysis revealed a significant positive relationship ($r = 0.60$, $P < 0.02$) between glucose disposal rates and PI3-kinase activation (Fig. 3). PI3-kinase activation was also significantly related to $\dot{V}O_{2\,max}$ ($r = 0.53$, $P < 0.04$). There

Table 2. *Glucose and insulin concentrations at rest and during 90–120 min of euglycemic-hyperinsulinemic clamps for the sedentary and exercise-trained subjects*

Variable	Sedentary	Trained
Glucose, mM		
Basal	5.4 ± 0.1	5.3 ± 0.1
Clamp	4.9 ± 0.1	5.1 ± 0.1
CV, %	4.8 ± 0.5	5.6 ± 0.7
Insulin, pM		
Basal	54 ± 4	51 ± 3
Clamp	314 ± 25	293 ± 21

Values are means ± SE; $n = 8$ in each group. Basal: average of −30-, −20-, −10-, and 0-min baseline samples. Clamp: average of 90- to 120-min samples. CV, coefficient of variation.

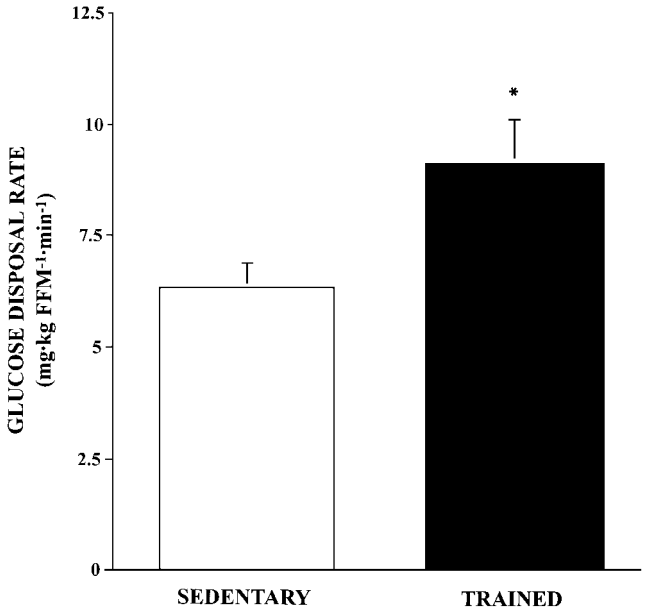

Fig. 1. Mean glucose disposal rates during euglycemic-hyperinsulinemic clamps performed on a group of exercise-trained and healthy sedentary subjects. FFM, fat-free mass. *Significant difference between groups, $P < 0.05$.

was no correlation between PI3-kinase activation and capillary density ($r = 0.04$, $P = 0.93$) or glucose disposal rate and capillary density ($r = 0.51$, $P = 0.16$).

DISCUSSION

In the present study, we examined the role that regular exercise plays in altering the regulation of insulin-mediated glucose metabolism in skeletal muscle. The results show that individuals with a high level of physical conditioning have greater insulin-stimulated IRS-1-associated PI3-kinase activation in skeletal muscle during hyperinsulinemia. The enhanced PI3-kinase activation is directly associated with augmented insulin-mediated glucose disposal and $\dot{V}O_{2max}$, both of which are characteristic of physically fit individuals. Thus a key step in the intracellular insulin signaling pathway is upregulated at a time when insulin-mediated glucose transport by skeletal muscle is also increased relative to sedentary control subjects. These results extend our knowledge of the potential mechanisms that underlie exercise-induced improvements in insulin-mediated glucose transport in human skeletal muscle in vivo.

In our present study, the benefit of enhanced physical conditioning on insulin-mediated glucose uptake was evidenced by the greater rate of glucose disposal (31%) in skeletal muscle of the exercise trained vs. sedentary subjects. These data are consistent with the work of other investigators who have reported 20–30% differences in glucose uptake during hyperinsulinemia when comparing trained and untrained individuals (26, 34). Upregulation of the molecular steps in the insulin signaling pathway may play a primary role in facilitating the observed increase in insulin-mediated glucose uptake. Kim et al. (24) have shown that exercise training can bring about a significant increase in insulin receptor, IRS-1, and mitogen-activated protein kinase mRNA levels in murine skeletal muscle. In the present study, we examined PI3-kinase activation because it appears to be an important protein in the insulin signaling cascade. Furthermore, it has been shown that activation of PI3-kinase is essential for GLUT-4 translocation and insulin-mediated glucose uptake in skeletal muscle (31, 32). The relative importance of PI3-kinase as a regulatory step in the insulin signaling pathway is further underscored by data showing an association between decreased PI3-kinase activation and reduced glucose transport ability in studies using human and animal models of diabetes, insulin resistance, and obesity (4, 16, 20). To date, there are no data on the effects of exercise training on insulin-stimulated PI3-kinase activity in human skeletal muscle. However, Han et al. (18) have reported preliminary data showing increases in IRS-1-associated PI3-kinase activity in rats undergoing voluntary exercise on a running wheel. The present study provides the first evidence showing an increase in insulin activation of IRS-1-associated PI3-kinase activity in human skeletal muscle with regular exercise. On the basis of the combined data from human and animal models, it appears that the ability to upregulate PI3-kinase through a program of regular exercise may serve to facilitate greater insulin-mediated glucose uptake.

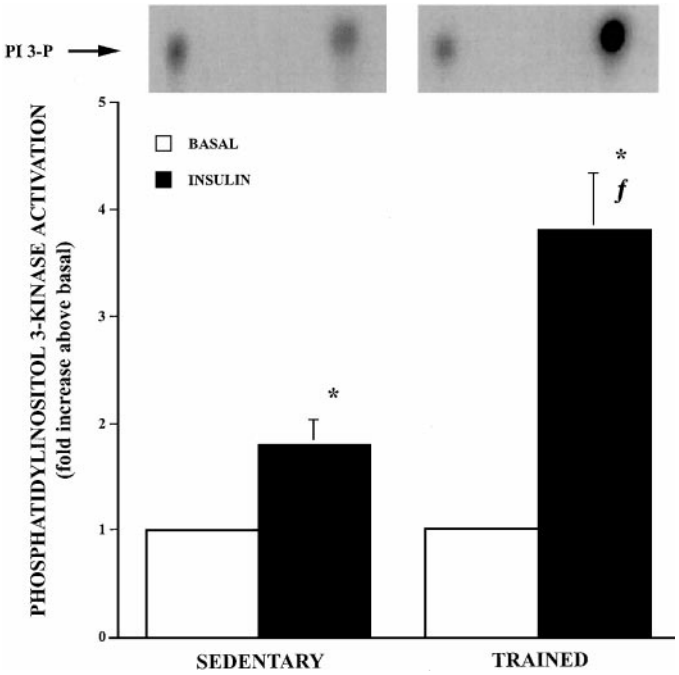

Fig. 2. Phosphatidylinositol 3-kinase (PI3-kinase) activation in the vastus lateralis muscle under fasting basal conditions and after 2 h of hyperinsulinemia. *Top*: representative immunoblot for 1 sedentary and 1 exercise-trained subject. A 1-mg sample of solubilized protein was immunoprecipitated with an insulin receptor substrate-1 (IRS-1) antibody as described in METHODS. Radioactivity incorporated into PI3-phosphate (PI3-P) was determined by phosphorimaging. Data in *bottom* represent means ± SE; $n = 8$ per group. *Significant increase above basal, $P < 0.003$ for the sedentary group and $P < 0.0001$ for the trained group. *f* Significantly greater response in the exercise trained group compared with the sedentary group, $P < 0.004$.

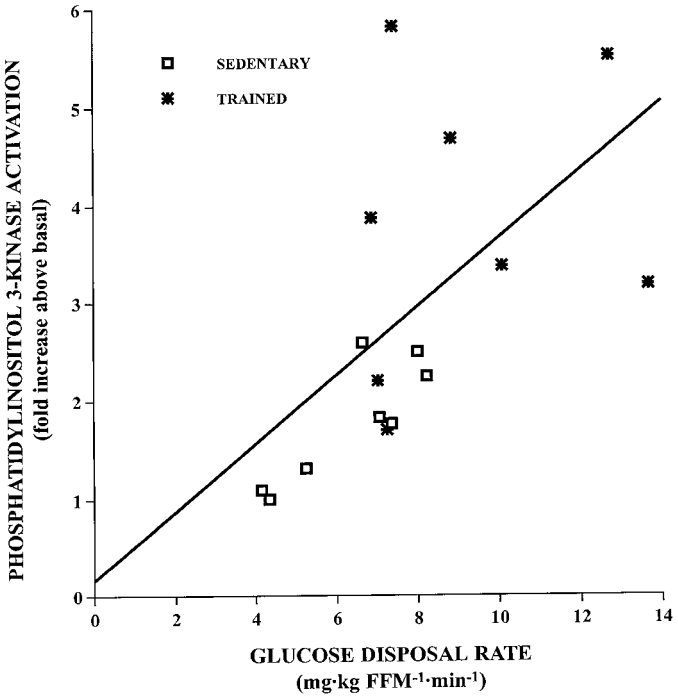

Fig. 3. Relationship between glucose disposal rate and skeletal muscle PI3-kinase activation after 2 h of hyperinsulinemia ($r = 0.60$, $P < 0.02$).

These data provide evidence at the molecular level to support the efficacy of exercise as an intervention in the treatment of insulin resistance and Type 2 diabetes in humans.

The trained group in this study was comprised of individuals who performed aerobic exercise on a regular basis and had been engaged in sport for most of their life. The $\dot{V}O_{2max}$ of the trained group was significantly higher than their sedentary counterparts, and correlation analysis revealed a significant positive relationship between $\dot{V}O_{2max}$ and PI3-kinase activation. Therefore, our data suggest a strong link between exercise capacity and the increased insulin signaling ability of the muscle obtained from the trained group. It is possible that the enhanced insulin action in the trained group may be due to elevated protein expression of steps in the insulin signaling pathway. Due to technical limitations, we were unable to measure PI3-kinase protein expression. However, we did use equal amounts of protein in the assays performed on muscle obtained from both groups. Thus, in the assay performed under the same conditions of protein in vitro, insulin-induced PI3-kinase activity is greater in the trained group. Further work is required to determine if the observed increase in PI3-kinase activity is accompanied by increased protein expression or perhaps by changes in more proximal steps in the pathway, e.g., increased insulin receptor phosphorylation or increased tyrosine phosphorylation of IRS-1.

Some of the metabolic adaptations associated with exercise training have been ascribed to the previous exercise session rather than long-term changes in metabolism. Measurements of insulin-mediated glucose uptake in trained subjects who have stopped exercising suggest that most of the exercise training effects are lost ~48 h after the cessation of exercise (25, 35). However, it has been shown that insulin-mediated leg glucose uptake rates are higher after 10 wk of exercise training than after a single exercise bout (10). Thus, whereas the effects of exercise may be transient, chronic exercise training appears to induce a physiological adaptation that augments insulin sensitivity in skeletal muscle. Furthermore, individuals who exercise regularly may maintain a higher metabolic rate, and this may help to sustain the benefits associated with exercise in a relatively permanent manner, assuming that the individual continues to perform exercise. In the present study, we performed our measurements of insulin-mediated glucose uptake and PI3-kinase activity ~18 h after the last exercise session for the trained group. Thus, whereas measurements made at this time may be influenced by the last exercise bout, it is perhaps more physiologically reflective of the insulin and metabolic milieu that is characteristic of individuals who exercise on a regular basis.

The effect of exercise training on insulin signaling in the present study should be distinguished from previous reports on the immediate effects of acute exercise on *1*) specific steps in the insulin signaling pathway and *2*) insulin stimulation of the insulin signaling pathway. There is no increase in insulin receptor autophosphorylation, tyrosine phosphorylation of the insulin receptor, or IRS-1 and PI3-kinase activation in human or animal muscle immediately after a single bout of exercise or after muscle contraction (16, 38, 40). Furthermore, it has been shown that muscle contraction can have a negative effect on insulin signaling and specifically on PI3-kinase activation in rat epitroclearis muscle (15). Data from our laboratory also show that, in well-trained athletes, an acute bout of intense cycling exercise to exhaustion causes a decrease in IRS-1-associated PI3-kinase for up to 30 min after the exercise bout (12). Likewise, insulin-stimulated IRS-1-associated PI3-kinase is also reduced in human skeletal muscle for up to 5 h after a single bout of exercise (40). In contrast, Zhou and Dohm (43) reported an increase in insulin-stimulated PI3-kinase activity in rat muscle after a single bout of treadmill running. These later findings raise interesting questions regarding the specific methodologies employed to immunoprecipitate PI3-kinase. Zhou and Dohm (43) used an anti-phosphotyrosine antibody, which immunoprecipitates both IRS-1- and IRS-2-associated PI3-kinase. We and others have immunoprecipitated only the IRS-1-associated isoform. After an acute bout of exercise, IRS-2-associated PI3-kinase may be increased in compensation for downregulation in the IRS-1-associated isoform. In contrast, exercise training brings about adaptations at the molecular level that include an increase in IRS-1-associated PI3-kinase. At this time, we do not know if there is also an increase in IRS-2-associated PI3-kinase. The reasons for the apparent conflicting observations between acute exercise and exercise training are unclear at present but may be related to competing biochemical and

metabolic demands during the initial recovery from an acute bout of exercise as opposed to the relatively stable metabolic state that is present 12–18 h after the exercise session. The reason for the conflicting data on the effects of acute exercise on insulin-stimulated PI3-kinase may be related to activation of alternate signaling pathways, which have not been fully examined in studies to date. It remains to be determined whether these alternative insulin signaling pathways are downregulated by exercise or muscle contraction alone.

Apart from insulin receptor and/or postreceptor events, improved insulin-mediated glucose metabolism after exercise training could be determined by a prereceptor or a central adaptive response. Changes in hemodynamics leading to greater insulin and glucose delivery to the muscle have been noted when exercise is combined with insulin infusion (5, 8). Augmented basal and exercise-induced blood flow is a key adaptation associated with physical training. A significant relationship has been shown between basal limb blood flow and insulin-mediated glucose disposal in athletes (14). In addition, insulin transport across the capillary appears to be rate limiting for insulin action (36, 41). We did not see any significant differences in muscle capillary density in the trained vs. sedentary subjects, although the capillary-to-fiber ratio was ~56% higher ($P = 0.07$) in the trained group. The trend toward a potentially greater ability to perfuse the muscle in the trained group may have contributed to a slightly greater delivery of insulin to the receptor site on the plasma membrane of the muscle cell. However, our data are not conclusive on this point. Thus the effects of exercise training on the contribution of increased insulin and glucose delivery to the muscle and in turn overall increases in insulin-mediated glucose uptake remain to be determined.

In conclusion, the enhanced insulin sensitivity associated with exercise training appears to be mediated by increased postreceptor insulin signaling, specifically at the IRS-1-associated PI3-kinase step in the cascade that leads to GLUT-4 translocation and glucose uptake. These data strengthen the basis for recommending exercise as a therapeutic intervention that can directly improve insulin action on skeletal muscle among patients with insulin resistance and Type 2 diabetes. The present study also provides new and important data elucidating the molecular basis for the effects of physical conditioning on insulin action in human skeletal muscle.

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