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Insulin-stimulated insulin receptor substrate-2–associated phosphatidylinositol 3–kinase activity is enhanced in human skeletal muscle after exercise

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Abstract

Exercise increases skeletal muscle insulin action but the underlying mechanisms mediating this are equivocal. In mouse skeletal muscle, prior exercise enhances insulin-stimulated insulin receptor substrate-2 (IRS-2) signaling (*Diabetes* 2002;51:479-83), but it is unknown if this also occurs in humans. Hyperinsulinemic-euglycemic clamps were performed on 7 untrained males at rest and immediately after 60 minutes of cycling exercise at ~75% Vo_{2peak}. Muscle biopsies were obtained at basal, immediately after exercise, and at 30 and 120 minutes of hyperinsulinemia. Insulin infusion increased ($P < .05$) insulin receptor tyrosine phosphorylation similarly in both the rest and exercise trials. Under resting conditions, insulin infusion resulted in a small, but non–statistically significant increase in IRS-2–associated phosphatidylinositol 3 (PI 3)–kinase activity over basal levels. Exercise per se decreased ($P < .05$) IRS-2–associated PI 3–kinase activity. After exercise, insulin-stimulated IRS-2–associated PI 3–kinase activity tended to increase at 30 minutes and further increased ($P < .05$) at 120 minutes when compared with the resting trial. Insulin increased ($P < .05$) Akt Ser^{473} and GSK-3α/β Ser^{21}/Ser^{9} phosphorylation in both trials, with the response tending to be higher in the exercise trial. In conclusion, in the immediate period after an acute bout of exercise, insulin-stimulated IRS-2 signaling is enhanced in human skeletal muscle.

1. Introduction

It is well established that exercise can enhance the metabolic action of insulin [1-3]. As such, exercise is an important intervention strategy for the maintenance of good health, and the prevention and treatment of skeletal muscle insulin resistance and type 2 diabetes mellitus (non–insulin-dependent diabetes mellitus) [4-6]. The underlying mechanisms responsible for the beneficial effects of exercise on insulin action are equivocal; but it has previously been demonstrated that exercise may affect skeletal muscle insulin action by influencing specific events in the insulin signaling pathway, in particular at the level of insulin receptor substrate (IRS) proteins [7,8].

Insulin receptor substrate proteins are key mediators in insulin signaling, where they act as docking proteins between the insulin receptor and a complex network of downstream signaling pathways, including phosphatidylinositol 3–kinase (PI 3–kinase) [9]. The IRS proteins, in particular IRS-1 and IRS-2, which are predominantly expressed in skeletal muscle, play a central role in mediating many of the normal metabolic actions of insulin. Furthermore, defects at the level of the IRS proteins comprise a major locus for the development of metabolic disorders including insulin resistance and type 2 diabetes mellitus [10-12].

The effect of exercise or muscle contraction on insulin-stimulated IRS-1 signaling has been examined in both humans [13,14] and rodents [15,16], with the majority of studies demonstrating in the period after exercise (<4 hours postexercise) a decrease in insulin-stimulated IRS-1–associated PI 3–kinase activity compared with insulin stimulation alone [14-16]. In contrast, few studies have examined

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the effect of exercise or muscle contraction on IRS-2 signaling. Insulin-stimulated IRS-2–associated PI 3–kinase activity has been demonstrated to be inhibited with ex vivo contractions in rat epitrochlearis muscle [16], whereas in response to in vivo exercise insulin-stimulated IRS-2–associated PI 3–kinase activity was markedly enhanced in mouse skeletal muscle [7]. These conflicting findings could be accounted for by differences in the exercise or contractile model used. Despite these limited studies in rodent skeletal muscle, to our knowledge, no study has determined the effect of exercise on insulin-stimulated IRS-2 signaling in human skeletal muscle. Thus, the aim of the present study was to examine IRS-2 signaling in human skeletal muscle during a hyperinsulinemic-euglycemic clamp in the immediate period after an acute bout of aerobic exercise.

2. Methods

2.1. Subjects

Seven active, but untrained, healthy males (24 ± 2 years, 73 ± 3 kg, mean ± SEM) volunteered to serve as subjects for the experiment. The experimental procedures and possible risks of the study were explained to each subject verbally and in writing. All subjects gave their informed, written consent, and the experiment was approved by the Deakin University Human Research Ethics Committee.

2.2. Preexperimental protocol

All subjects performed an incremental workload test to exhaustion on an electromagnetically braked cycle ergometer (LODE Instrument, Groningen, The Netherlands) to determine their peak pulmonary oxygen uptake ($V\dot{O}_{2peak}$). Oxygen uptake was measured using an online system and calculated using Vista/Turbofit software (Vacumetrics, Ventura, CA). Expired oxygen and carbon dioxide were measured with zirconia cell oxygen and infrared carbon dioxide analyzers (AEI Technologies, Pittsburgh, PA) that were calibrated with standard commercial gases. Ventilation was measured by a turbine flow transducer (KL Engineering, Van Nuys, CA). Peak $V\dot{O}_2$ was the highest $V\dot{O}_2$ attained during the latter stages of the test and was accompanied by a respiratory exchange ratio (RER) that was greater than 1.1. Mean $V\dot{O}_{2peak}$ was 3.63 ± 0.22 L min^{-1}. For the day preceding each trial, the subjects consumed a food package (~14 MJ, 80% total energy as carbohydrate) and abstained from exercise, tobacco, caffeine, and alcohol. In addition, they were instructed to consume ~5 mL of tap water per kg body weight upon waking to ensure adequate hydration. The subjects reported to the laboratory in the morning after a 10- to 12-hour overnight fast.

2.3. Experimental protocol

Subjects undertook 2 experimental trials that were performed in a random cross-over design and separated by at least a week. Subjects either rested on a bed or exercised for 60 minutes at a power output eliciting 75% ± 4% $V\dot{O}_{2peak}$. During exercise, expired gases were sampled for measurement of $V\dot{O}_2$, ventilation, and RER as described above. Heart rate was measured continuously using a heart rate monitor (Polar Electro, Kempere, Finland). In the immediate period after the rest or exercise trial, a hyperinsulinemic-euglycemic clamp was initiated by an intravenous bolus injection of insulin (9 mU kg^{-1}). Insulin was then constantly infused at a rate of 40 mU m^{-2} min^{-1} for 120 minutes into an arm vein, whereas glucose was variably infused in the contralateral arm vein. From a separate hand vein, blood samples that had been arterialized by hand warming were obtained every 5 minutes during the clamp for measurement of glucose, and the glucose infusion was adjusted to maintain blood glucose at a constant value of 5 mmol L^{-1}. Before each experimental trial, the subjects were administered with an oral dose of 30 mmol of KCl (Slow K, Novartis, North Ryde, Australia) to maintain plasma potassium levels. Additional blood samples were taken before and during exercise, and at regular intervals during the hyperinsulinemic-euglycemic clamp for analysis of blood lactate, and plasma insulin and free fatty acids (FFAs). Muscle samples were obtained from the vastus lateralis, by the percutaneous needle biopsy technique modified for suction [17], at basal, immediately postexercise, after 30 minutes, and at the completion of the hyperinsulinemic-euglycemic clamp. The muscle samples were immediately frozen in liquid nitrogen for later analysis.

2.4. Blood and muscle glycogen analysis

Blood glucose and lactate were measured using an automated glucose-lactate analyzer (EML 105 Radiometer, Copenhagen, Denmark). Plasma insulin was measured by radioimmunoassay (Human Insulin Specific RIA Kit, Linco Research, St Charles, Mo). Plasma FFAs were measured by an enzymatic colorimetric method (Wako NEFA C test kit, Wako Chemicals, Osaka, Japan). For measurement of muscle glycogen concentrations, muscle was freeze dried, dissected free of visible connective tissue and blood, and then powdered. Glycogen content was determined as previously described [18].

2.5. Skeletal muscle tissue processing

Muscle was homogenized in either buffer A (50 mmol/L HEPES, 150 mmol/L NaCl, 20 mmol/L $Na_4P_2O_7$, 20 mmol/L β-glycerolphosphate, 10 mmol/L NaF, 2 mmol/L Na_3VO_4, 2 mmol/L EDTA, 1% Igepal, 10% glycerol, 2 mmol/L PMSF, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 μg mL^{-1} leupeptin, 10 μg mL^{-1} aprotinin, 3 mmol/L benzamidine) or buffer (20 mmol/L HEPES, 50 mmol/L β-glycerolphosphate, 1 mmol/L Na_3VO_4, 2 mmol/L EGTA, 1% Triton X-100, 10% glycerol, 1 mmol/L DTT, 1 mmol/L PMSF, 10 μmol/L leupeptin, 10 μg mL^{-1} aprotinin, 3 mmol/L benzamidine, 5 μmol/L pepstatin A) and rotated end over end for 1 hour at 4°C. Homogenates were spun at 15 000 × g for 1 hour, the

Table 1
Hormonal and metabolic responses during 60 min of exercise

	Rest	20 min	40 min	60 min
Insulin (pmol L^{-1})	56 ± 4	52 ± 3	54 ± 9	42 ± 9
Glucose (mmol L^{-1})	4.6 ± 0.1	4.9 ± 0.4	4.8 ± 0.4	4.7 ± 0.4
Lactate (mmol L^{-1})	1.3 ± 0.2	7.1 ± 1.5*	5.9 ± 1.1*	4.7 ± 0.7*
FFA (mmol L^{-1})	0.37 ± 0.06	0.24 ± 0.03	0.29 ± 0.04	0.44 ± 0.06
Muscle glycogen (mmol kg^{-1} dw)	449 ± 66	NA	NA	197 ± 71*

Data are mean ± SEM (n = 7). dw indicates dry weight; NA, no measurement.
* $P < .05$ compared with rest.

supernatants were collected, and aliquots were stored at −80°C for later analysis. Total protein concentrations were determined (BCA Assay Kit, Pierce, Rockford, IL) using bovine serum albumin (BSA) as the standard.

2.6. Activity assays

Equal amounts of muscle protein (1000 μg, Buffer A) were immunoprecipitated overnight at 4°C with a polyclonal anti–IRS-2 antibody (Upstate Biotechnology, Lake Placid, NY). Immune complexes were then bound to protein A Sepharose and washed extensively. Phosphatidylinositol 3–kinase activity was assayed as described previously [15]. [^{32}P] incorporation was quantified using a Phosphoimager (Molecular Dynamics, Sunnyvale, CA).

2.7. Immunoblotting

Equal amounts of muscle protein (60 μg, Buffer B) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (8% or 10% polyacrylamide) and transferred to nitrocellulose membranes at 100 V for 90 minutes. Membranes were incubated for 1 hour at room temperature in blocking buffer (5% BSA in Tris-buffered saline with 0.5% Tween [TBST]) and then overnight at 4°C in polyclonal antibodies in buffer (5% BSA in TBST) that recognize IRS-2 (Upstate Biotechnology), phosphorylated Akt (Ser^{473}), and GSK-3α/β (Ser^{21}/Ser^{9}) (Cell Signaling Technology, Beverly, MA). Membranes were exposed to horseradish peroxidase–conjugated secondary antibody (1:2000) in buffer (5% nonfat milk in TBST) for 60 minutes. Antibody binding was viewed by enhanced chemiluminescence substrate (Pierce SuperSignal Chemiluminescent, Pierce) and a Kodak Image Station 440CF (NEN Life Science Products, Boston, MA). Bands were identified and quantified using Kodak 1D Image Analysis Software (Eastman Kodak, Rochester, NY).

For tyrosine phosphorylation of the insulin receptor, equal amounts of muscle protein (300 μg, buffer B) were immunoprecipitated overnight at 4°C with anti-insulin receptor β subunit (Upstate Biotechnology). Immune complexes were bound to protein A Sepharose, washed, and then resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (8% polyacrylamide) and transferred to nitrocellulose membranes. Membranes were blocked and then incubated overnight at 4°C with anti-phosphotyrosine antibody (pY99, Santa Cruz Biotechnology, Santa Cruz, CA). Antibody binding was viewed as described above.

2.8. Calculation and statistical analysis

Standards were included in all kinase assays and immunoblotting, and interassay variation was accounted for by normalizing data to control samples. When the basal measurements between the experimental trials were not statistically different, the data were graphically presented as

Table 2
Hormonal and metabolic responses during a hyperinsulinaemic-euglycaemic clamp after rest or exercise

	0 min	30 min	60 min	90 min	120 min
Insulin (pmol L^{-1})					
Rest	79 ± 15	646 ± 33	666 ± 18	743 ± 60	736 ± 53†
Exercise	107 ± 26	683 ± 46	737 ± 53	703 ± 71	719 ± 46†
Glucose (mmol L^{-1})					
Rest	4.6 ± 0.2	4.8 ± 0.2	5.1 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
Exercise	4.4 ± 0.1	4.9 ± 0.1	5.1 ± 0.3	5.1 ± 0.1	5.0 ± 0.1
Lactate (mmol L^{-1})					
Rest	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
Exercise	2.4 ± 0.3*	1.7 ± 0.2*	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
FFA (mmol L^{-1})					
Rest	0.39 ± 0.07	0.15 ± 0.02	0.12 ± 0.01	0.09 ± 0.01	0.09 ± 0.01†
Exercise	0.62 ± 0.14	0.22 ± 0.04	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01†
Muscle glycogen (mmol kg^{-1} dw)					
Rest	409 ± 52	385 ± 51	NA	NA	435 ± 50‡
Exercise	197 ± 71	217 ± 65	NA	NA	200 ± 45

Data are mean ± SEM (n = 7).
* $P < .05$ compared with rest.
† $P < .05$ time effect.
‡ $P < .05$ treatment effect.

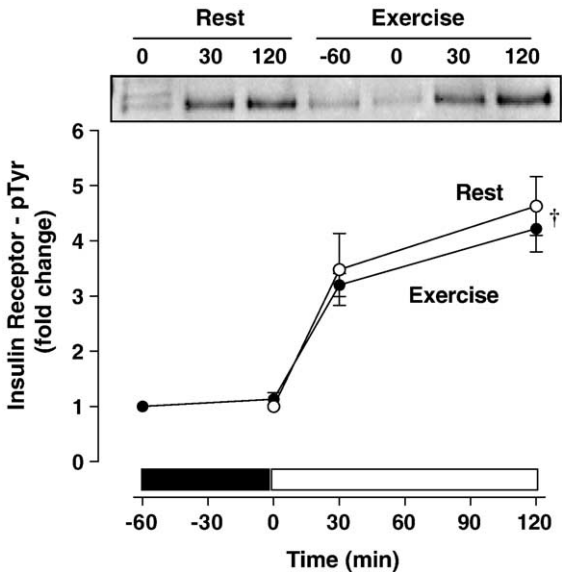

Fig. 1. Insulin receptor tyrosine phosphorylation in response to a hyperinsulinaemic-euglycaemic clamp at rest and after exercise. Data are representative blots and mean ± SEM (n = 7). Black bar indicates exercise; white bar, hyperinsulinaemic-euglycaemic clamp; p, phosphorylation; Tyr, tyrosine residue. †$P < .05$ time effect.

fold change. All data are expressed as mean ± SEM. Statistical analysis was undertaken using Student *t* test and 2-way analysis of variance. When the analysis of variance revealed significant differences, further analysis was performed using Student-Newman-Keuls post hoc test. The level of significance was set at $P < .05$.

3. Results

3.1. Exercise response

For the exercise trial, the subjects cycled for 60 minutes at a power output of 155 ± 11 W, which corresponded to 75 ± 4 %VO_{2peak}. During exercise, the subjects had an average heart rate of 161 ± 6 beats per minute; RER, 0.90 ± 0.02; and ventilation, 64 ± 7 L min^{-1}. There was a significant increase in blood lactate during exercise, whereas there was no significant change in blood glucose, plasma FFA, and insulin (Table 1). Muscle glycogen decreased significantly during exercise (Table 1).

3.2. Hyperinsulinemic-euglycemic clamp

Infusion of insulin during the hyperinsulinemic-euglycemic clamp significantly increased ($P < .05$ time effect) plasma insulin to high physiological levels in both the rest and exercise trials (Table 2). In each trial, exogenous glucose was variably infused such that blood glucose levels remained at ~5 mmol L^{-1} for the duration of the clamp (Table 2). The rate of glucose infusion throughout the duration of the hyperinsulinemic-euglycemic clamp was similar after a rest period or exercise (data not shown). The average glucose infusion rates during the final 30 minutes of the clamp were 9.1 ± 1.1 and 8.3 ± 1.0 mg kg^{-1} min^{-1} in the rest and exercise trial, respectively.

Blood lactate levels during the first 30 minutes of the hyperinsulinemic-euglycemic clamp remained elevated in the exercise trial compared with the rest trial, but by 60 minutes of the clamp blood lactate had returned to pre-exercise levels and was similar to that in the resting trial (Table 2). Before the commencement of the clamp, there was a tendency for plasma FFA to be higher in the exercise trial (Table 2). However, infusion of insulin during the clamp resulted in a significant ($P < .05$ time effect) decrease in plasma FFA levels in both the rest and exercise trials. Muscle glycogen levels were similar in both trials at basal (rest, 409 ± 52; exercise, 449 ± 66 mmol kg^{-1} dry weight). Muscle glycogen levels were reduced ($P < .05$) during exercise and remained at significantly lower ($P < .05$ treatment effect) levels compared with the resting trial for the duration of the hyperinsulinemic-euglycemic clamp (Table 2).

3.3. Insulin signaling proteins

Exercise had no effect on insulin receptor tyrosine phosphorylation (Fig. 1). Insulin infusion resulted in significant increase ($P < .05$ time effect) in insulin receptor tyrosine phosphorylation that was similar after either a rest period or exercise.

Basal measurements for IRS-2–associated PI 3–kinase activity were similar in both the rest and exercise trials (rest, 2.4 ± 0.5; exercise, 1.8 ± 0.5 arbitrary units). In the resting trial, in response to insulin infusion there was a small, but non–statistically significant, increase in IRS-2–

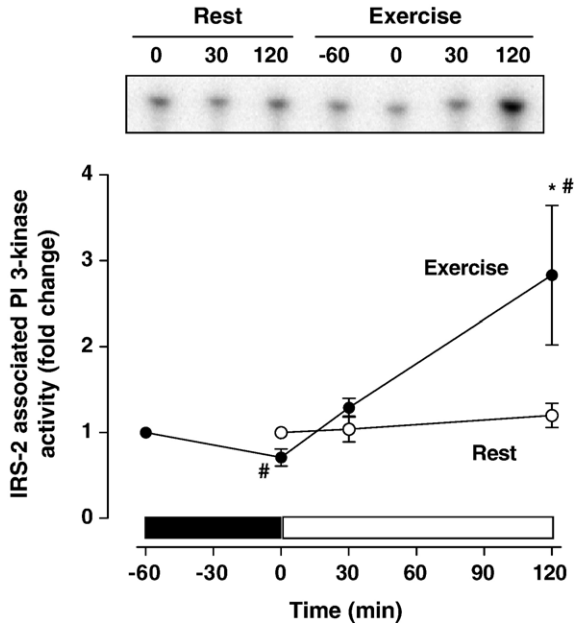

Fig. 2. IRS-2–associated PI 3–kinase activity in response to a hyperinsulinaemic-euglycaemic clamp at rest and after exercise. Data are representative blots and mean ± SEM (n = 7). Black bar indicates exercise; white bar, hyperinsulinaemic-euglycaemic clamp. *$P < .05$ compared with rest. #$P < .05$ compared with −60 minutes.

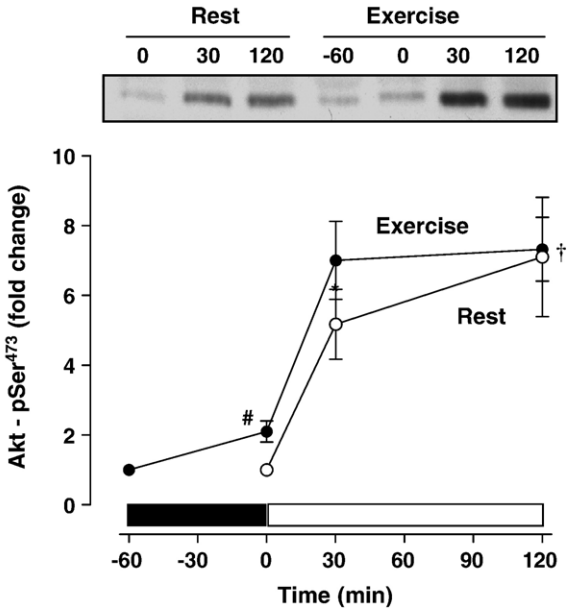

Fig. 3. Akt Ser^{473} phosphorylation in response to a hyperinsulinaemic-euglycaemic clamp at rest and after exercise. Data are representative blots and mean ± SEM (n = 7). Black bar indicates, exercise; white bar, hyperinsulinaemic-euglycaemic clamp; p, phosphorylation; Ser, serine residue. $^{\#}P < .05$ compared with −60 minutes. †$P < .05$ time effect.

associated PI 3–kinase activity over basal levels (Fig. 2). Exercise resulted in an approximately 30% decrease in IRS-2–associated PI 3–kinase activity ($P < .05$) (Fig. 2). However, in the immediate period after exercise insulin stimulation resulted in a marked increase in IRS-2–associated PI 3–kinase activity that was significantly greater when compared with insulin stimulation during the resting trial (Fig. 2). The exercise-mediated increase in insulin-stimulated IRS-2–associated PI 3–kinase activity (Fig. 2) was relatively small at 30 minutes, but further increased ($P < .05$) at 120 minutes of the clamp. Changes in IRS-2–associated PI 3–kinase activity were not due to differences in IRS-2 protein levels, as these measurements were similar in both the rest (basal, 1.2 ± 0.2; 30 minutes, 1.7 ± 0.6; and 120 minutes, 1.3 ± 0.4 arbitrary units) and exercise (basal, 1.8 ± 0.8; postexercise, 1.2 ± 0.4; 30 minutes, 1.6 ± 0.4; and 120 minutes, 1.7 ± 0.5 arbitrary units) trials.

Basal measurements for phosphorylated Akt Ser^{473} (rest, 1.4 ± 0.3; exercise, 1.3 ± 0.3 arbitrary units), GSK-3α Ser^{21} (rest, 4.7 ± 0.6; exercise, 3.3 ± 0.6 arbitrary units) and GSK-3β Ser^{9} (rest, 1.6 ± 0.1; exercise, 1.4 ± 0.5 arbitrary units) were similar in both the rest and exercise trials. Phosphorylation of Akt Ser^{473} was increased ($P < .05$) by approximately 110% after 60 minutes of exercise (Fig. 3). Insulin infusion in the immediate period after exercise resulted in a further increase in Akt Ser^{473} phosphorylation ($P < .05$ time effect); however, the increase was similar when compared with insulin stimulation alone. GSK-3α Ser^{21} and GSK-3β Ser^{9} phosphorylation were not significantly increased after exercise (Fig. 4A and B). During the hyperinsulinemic-euglycemic clamp, there was an increase ($P < .05$ time effect) in both GSK-3α Ser^{21} and GSK-3β Ser^{9} phosphorylation. In the exercise trial, insulin-stimulated GSK-3α Ser^{21} phosphorylation appeared to be greater, but when compared with insulin stimulation alone neither phosphorylation of GSK-3α Ser^{21} nor that of GSK-3β Ser^{9} was significantly different.

4. Discussion

In the present study, we have shown that in response to a hyperinsulinemic-euglycemic clamp a prior acute bout of aerobic exercise can enhance IRS-2–associated PI 3–kinase activity in human skeletal muscle. The finding that insulin-stimulated IRS-2–associated PI 3–kinase activity is in-

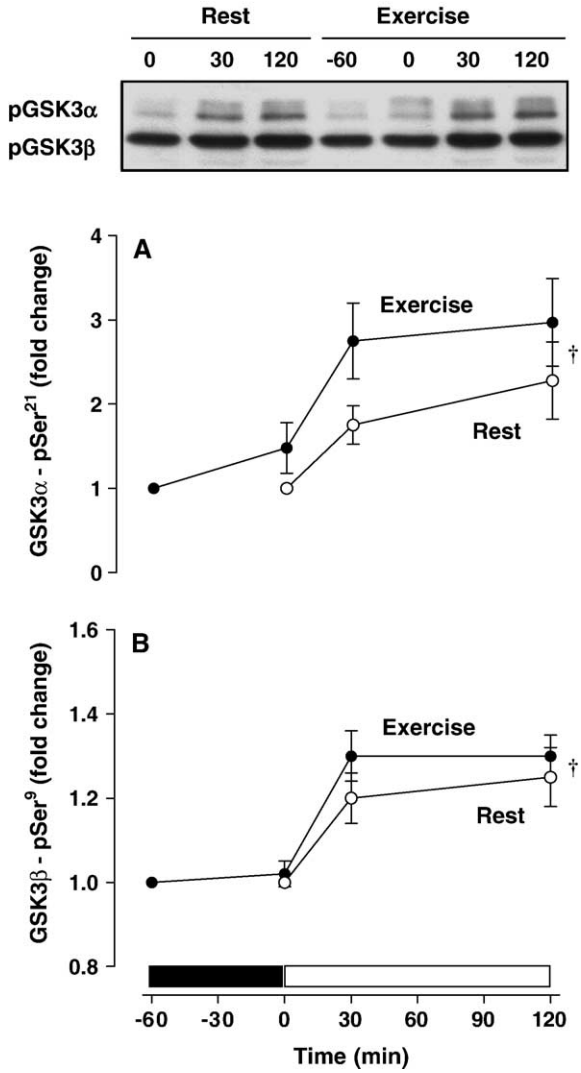

Fig. 4. Glycogen synthase kinase-3α Ser^{21} (A) and GSK-3β Ser^{9} (B) phosphorylation in response to a hyperinsulinaemic-euglycaemic clamp at rest and after exercise. Data are representative blots and mean ± SEM (n = 7). Black bar indicates exercise; white bar, hyperinsulinaemic-euglycaemic clamp; p, phosphorylation; Ser, serine residue. †$P < .05$ time effect.

creased in the immediate period after exercise compared with insulin stimulation alone is the first time, to our knowledge, that this has been demonstrated in humans. This finding supports previous results in mouse skeletal muscle which demonstrate that IRS-2 is an important tyrosine phosphoprotein that can mediate enhanced insulin-stimulated PI 3–kinase activity in the immediate period after exercise [7].

The underlying mechanism(s) mediating the increase in insulin-stimulated IRS-2–associated PI 3–kinase activity after exercise is unknown, although the exercise-mediated effects on IRS-2 insulin signaling are unlikely to be due to enhanced insulin receptor binding or activation as insulin-stimulated insulin receptor tyrosine phosphorylation is not altered by exercise [14,16,19] (Fig. 1). In some cell systems, changes in the subcellular localization of IRS proteins can play an important role in determining the specificity and magnitude of IRS-mediated insulin signaling [20,21]. However, a recent study demonstrates that exercise does not result in changes in the subcellular localization of IRS proteins in human skeletal muscle, and it is therefore an unlikely mechanism to account for the increase in insulin-stimulated IRS-2 signaling after exercise [22]. It is possible that the increase in insulin-stimulated IRS-2–associated PI 3–kinase activity after exercise may be due to the activation and interaction of other proteins and/or kinases that are associated with exercise-induced changes in energy status, fuel depletion, tension development, and/or muscle fatigue [23]. A number of protein kinases have been identified that can attenuate or augment insulin signaling by phosphorylating specific IRS tyrosine and/or serine residues, although the majority of studies have focused on the regulation of IRS-1 rather than IRS-2 [24]. Many of these protein kinases (eg, protein kinase B/Akt, c-Jun NH2 kinase, and adenosine monophosphate–activated protein kinase) are also activated by exercise or muscle contraction [25-27] (Fig. 3). However, it remains to be determined whether exercise-mediated activation of these protein kinases influences the activation of IRS-2 and whether this could account for the increase in insulin-stimulated IRS-2–associated PI 3–kinase activity after exercise.

In the present study, the increase in insulin stimulated IRS-2–associated PI 3–kinase activity after exercise did not result in a concomitant increase in insulin action as measured by whole-body glucose infusion rate during the hyperinsulinemic-euglycemic clamp. Although it is well established that an acute bout of exercise can enhance the metabolic action of insulin, and that the effects can persist for up to 48 hours postexercise [2], other studies have shown that when measured immediately after exercise, insulin action is not always enhanced [5,28]. In contrast, when measured longer than 3 to 4 hours postexercise in humans, improvements in insulin action have been detected despite minimal or no changes in proximal insulin signaling events [14,29]. These findings suggest that exercise/muscle contraction may differentially regulate insulin signaling and action. Alternatively, there may be delays in exercise-mediated activation of proximal insulin signaling events toward the downstream biological actions of insulin, with the results dependent on the period of time elapsed between the completion of exercise and when the measurements are performed.

The exact downstream physiological effect(s) of enhanced insulin-stimulated IRS-2–associated PI 3–kinase activity after exercise could not be determined in the present study. However, studies in IRS-2–deficient mice suggest that glucose transport is not impaired in isolated skeletal muscle after insulin and/or exercise [7,30], although under resting conditions IRS-2–deficient mice demonstrate impaired peripheral insulin signaling and a marked reduction in insulin-stimulated whole-body glucose utilization [11,12]. Alternatively, it has been suggested that IRS-2 may play a more important role in skeletal muscle by mediating glycogen synthesis rather than glucose uptake [11,31]. IRS-2–deficient mice have impaired insulin-stimulated muscle glycogen synthesis [11], and in L6 skeletal muscle cells expressing the $Arg^{1152} \rightarrow Gln$ insulin receptor mutation, IRS-2 activation was found to correlate significantly with glycogen synthesis and glycogen synthase activity [31]. Further research is required to determine whether IRS-2 plays a role in mediating the increase in insulin-stimulated glycogen synthase in human skeletal muscle after an acute bout of exercise [29].

It should be noted that the observation that an acute bout of exercise can enhance insulin stimulated IRS-2–associated PI 3–kinase activity in skeletal muscle of healthy untrained males may not necessarily apply to other populations such as females, the elderly, or those with obesity, insulin resistance, and/or type 2 diabetes. Studies examining IRS-2–mediated insulin signaling in human skeletal muscle are limited, but in individuals with type 2 diabetes, insulin-stimulated IRS-2–associated PI 3–kinase activity was reduced by ~39% [32], and IRS-2 association with the p85 subunit of PI 3–kinase was impaired [33] compared with control individuals. It is currently unknown as to whether a prior bout of acute exercise could improve insulin-stimulated IRS-2 signaling in these individuals or other populations.

In conclusion, we have shown that in the immediate period after an acute bout of exercise insulin-stimulated IRS-2–associated PI 3–kinase activity is enhanced in human skeletal muscle. Further research elucidating the underlying molecular mechanisms mediating improvements in skeletal muscle insulin signaling and the subsequent physiological effects will be important given the beneficial effects of exercise in the prevention and treatment of insulin resistance and type 2 diabetes mellitus.

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