Increased insulin-stimulated Akt pSer\textsuperscript{473} and cytosolic SHP2 protein abundance in human skeletal muscle following acute exercise and short-term training

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The serine-threonine kinase Akt [protein kinase B (PKB)] is an important signaling protein of many intracellular pathways, central for growth/muscle hypertrophy (17), insulin-stimulated glucose transport (20, 26), and protein and glycogen synthesis (28, 52). Akt activation is dependent on the phosphorylation of at least two key residues: Thr\textsuperscript{308} and Ser\textsuperscript{473} (4). In particular, phosphorylation of the Ser\textsuperscript{473} residue (pSer\textsuperscript{473}) is not only important for the phosphorylation of Thr\textsuperscript{308} (44) but necessary for the full activation of Akt kinase activity (44). Furthermore, pSer\textsuperscript{473} is often used in human (11, 22, 59) and rodent exercise studies (10, 34) to represent the degree of insulin-stimulated Akt activity. It has previously been demonstrated that reduced insulin-stimulated Akt pSer\textsuperscript{473} is evident in insulin-resistant obese individuals (7) and insulin-resistant HIV patients with lipodystrophy (19), although further studies have failed to demonstrate impairment in insulin-responsiveness of Akt pSer\textsuperscript{473} in either normal-weight first-degree relatives of diabetics or in individuals with Type 2 diabetes (24, 25). Despite these conflicting data, most (14, 22, 42, 57) but not all (51) human studies have shown that endurance exercise upregulates Akt pSer\textsuperscript{473} immediately postexercise. However, it is unclear whether exercise augments the insulin-stimulated increase in Akt activity and/or pSer\textsuperscript{473}Akt in human skeletal muscle. There is conflicting evidence for exercise increasing insulin-stimulated Akt pSer\textsuperscript{473} immediately following a single exercise bout (51, 59) and no increase after 8 wk of endurance training in people with Type 2 diabetes or in obese insulin-resistant controls (11). However, it is still not known what effect endurance training would have on insulin-stimulated Akt pSer\textsuperscript{473} in young, lean, and healthy humans. In rodents, insulin-stimulated Akt pSer\textsuperscript{473} is higher 24–48 h after 5 days and 6 wk of exercise training (10, 34) and reversed by detraining (32). Therefore, the first aim of the present study was to measure insulin-stimulated Akt pSer\textsuperscript{473} 24 h following both a single exercise bout and again following short-term (7 days) endurance exercise training in skeletal muscle from lean, young, healthy humans.
PTPases are key regulators of a wide variety of signal transduction pathways (46). Several PTPases have been identified to modulate the insulin signaling activity and hence are a therapeutic target for Type 2 diabetes (2, 16, 40, 62). PTP1B is an abundant PTPase within skeletal muscle (2) and a major mechanism negatively regulating (dephosphorylating) insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) activities (3, 8). Thus inhibition of PTP1B activity or PTP1B knockout enhances insulin sensitivity (16, 40, 62). Unlike most other PTPases, Src homology 2 (SH2) domain-containing PTP-2 (SHP2) has been implicated as a positive regulator of insulin signaling (9, 54) and myogenesis (30). The subcellular localization of PTPases could also be important since readily abundant PTPases in skeletal muscle and adipocytes each display characteristic subcellular distribution between the cytosol and particulate fractions (2, 8) and hence preferential sites of action depending on subcellular localization. Given the importance of PTPases in regulating insulin signaling, it is surprising that no data are available on the impact of exercise training.

Based on the findings in rodent skeletal muscle of increased insulin-stimulated Akt pSer473 following exercise (10, 34), we hypothesized that insulin-stimulated pSer473 Akt and tyrosine phosphorylation of IR and IRS-1 would be increased 24 h following acute exercise and exercise training in human skeletal muscle. Furthermore, since the protein abundance of PTPases is important in the regulation of the insulin-signaling pathway (16, 40, 62), we hypothesized that improvements in insulin sensitivity would be associated with altered abundance and/or subcellular distribution of either PTP1B or SHP2.

METHODS

Subjects. Eight untrained but healthy men (24 ± 1 yr, 82.3 ± 4.3 kg, 180 ± 3 cm, body mass index 25.6 ± 1.5 kg/m², means ± SE) volunteered to be involved in the study. None of the subjects had performed moderate- or vigorous-intensity exercise regularly (greater than once per week) in the past 12 mo. Subjects gave their written consent after all procedures and the possible risks of participation were explained. The experimental protocol and consent form were approved by the Deakin University Human Research Ethics Committee.

Subjects first visited the Exercise Muscle and Metabolism Unit, School of Exercise and Nutrition Sciences, Deakin University, for determination of peak pulmonary oxygen uptake rate (VO₂ peak) by an incremental exercise test to volitional fatigue on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands).Expired oxygen and carbon dioxide contents were measured with zirconia cell oxygen and infrared carbon dioxide analysers, respectively (AEI Technologies Pittsburgh, PA) that were calibrated with standard commercial gases. Ventilation was measured by a turbine flow transducer (KL Engineering California). VO₂ peak was the highest oxygen uptake (VO₂) attained during the latter stages of the test. The average subject VO₂ peak was 42.2 ± 1.6 ml·kg⁻¹·min⁻¹.

Experimental protocol. The experiment consisted of three trials where a hyperinsulinemic euglycemic clamp was performed J) in the nonexercised, rested state (rested), where subjects were instructed to only undertake normal levels of walking for the 48-h period before the clamp; 2) 24 h after an acute bout of exercise (acute); and then 3) 24 h after 7 days of consecutive exercise (trained). The resting trial was performed initially (day 1). Six days after the resting trial, subjects undertook an acute bout of exercise consisting of 60 min of cycling at 75% VO₂ peak (day 7). For the next 7 consecutive days (days 8–14), subjects cycled each day for 60 min at 75% VO₂ peak. During each of the exercise sessions, VO₂ was monitored and the workload was adjusted accordingly to maintain exercise intensity at 75% VO₂ peak. During the entire experimental period, subjects refrained from any physical activity other than that associated with the experimental protocol.

On the experimental days (days 1, 8, and 15) when the hyperinsulinemic euglycemic clamp was to be performed, subjects presented to laboratory in the morning after having fasted overnight for 10–12 h. Subjects were administered with an oral dose of 30 mmol of KCl to maintain plasma potassium levels. In the 24-h period before each experimental trial, subjects avoided the consumption of tobacco, alcohol, and caffeine. For 24 h before each experimental trial, subjects consumed a standardized diet (~14,500 kJ with 76, 12, and 12% of energy derived from carbohydrate, protein, and fat, respectively).

Hyperinsulinemic euglycemic clamp. A one-step hyperinsulinemic-euglycemic clamp was initiated by an intravenous bolus injection of insulin (9 mU/kg; Actrapid, Novo Nordisk, Denmark). Insulin was then constantly infused into an arm vein for 120 min at a rate of 40 μU·m⁻²·min⁻¹, while glucose was variably infused in the contralateral arm vein. From a separate warmed hand vein, blood samples were obtained every 5 min during the clamp for measurement of glucose (EML105, Radiometer Pacific, Melbourne, Australia), and the variable infusion rate of glucose was adjusted to maintain blood glucose at a constant value of 5 mM. Plasma insulin concentrations were determined in duplicate by radioimmunoassay using a commercially available kit (Phadephase, Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). Glucose infusion rate (GIR) was calculated as the average during the final 30 min of the clamp.

Skeletal muscle sampling. Muscle samples were obtained from the vastus lateralis by the percutaneous needle biopsy technique before the onset of each of the clamp and immediately at the completion of the hyperinsulinemic-euglycemic clamp. The muscle samples were immediately frozen in liquid nitrogen for later analysis.

Analytical materials. PTP1B mouse monoclonal IgG and SHP2 mouse monoclonal IgG were from BD Biosciences (San Diego, CA). IRS-1 rabbit polyclonal IgG was purchased from Upstate Biotechnology (New York, NY). Phosphotyrosine (PY99) mouse monoclonal IgG and IRS-1 (A-19) rabbit polyclonal IgG were from Santa Cruz Biotechnology. 83.7 IR mouse monoclonal IgG was a gift from Ken Siddle (University of Cambridge). Phospho-Akt (Akt pSer473) rabbit polyclonal IgG and Akt rabbit polyclonal IgG were from Cell Signaling Technology (New England BioLabs, Hartsfordshire, UK). Affinity purified peroxidase labeled anti-mouse IgG and anti-rabbit IgG were purchased from Silenus (Victoria, Australia). All other reagents were analytical grade (Sigma, NSW, Australia).

Preparation of whole muscle lysates. Frozen muscle (10 μl of buffer per mg of muscle) was homogenized using a polytron at maximum speed for 30 s in freshly prepared ice-cold buffer A [50 mM HEPES at pH 7.6 containing 150 mM NaCl, 20 mM Na₂HPO₄, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM EDTA, 1% vol/vol Nonidet P-40, 10% vol/vol glycerol, 1 mM MgCl₂, 1 mM CaCl₂, 2 mM Na₃VO₄, 2 mM PMSF, and 5 μl/ml protease inhibitor cocktail (P8340, Sigma)] (31). Tissue lysates were incubated on ice for 20 min and then spun at 10,000 g for 20 min at 4°C. Protein concentration was determined using the Bio-Rad protein assay (Bio-Rad, NSW, Australia) with BSA as the standard. The supernatants were stored at −80°C until analysis.

Preparation of fractionated muscle lysates. Frozen muscle (10 μl of buffer per mg of muscle) was homogenized using a polytron at maximum speed for 30 s in freshly prepared ice-cold buffer B [50 mM HEPES at pH 7.4 containing 1 mM DTT, 4 mM EDTA, 2.5 mM benzamidine, 2 mM PMSF, 5 μl/ml protease inhibitor cocktail (P8340, Sigma)]. The crude homogenate was spun at 350,000 g for 30 min at 4°C, and the resulting supernatant was taken as the cytosol fraction. Proteins in the particulate fraction were then solubilized by treating the pellet with ice-cold buffer B containing 1% vol/vol Triton X-100 and 0.2 M NaCl, incubating for 45 min at 4°C, then spun at 150,000 g for 60 min at 4°C. The resulting supernatant was taken as
the particulate fraction (33). Previously, using similar fractionation procedures to those employed here, we showed the cytosolic fraction to be devoid of membranes and the particulate fraction enriched in membranes (41). Protein concentration was determined using a bicinechonic protein assay (Pierce) with BSA as the standard. The supernatants were stored at −80°C until analysis.

**Immuno precipitation and -blotting.** For immunoprecipitation, anti-IRS-1 (A-19) polyclonal antibody (Santa Cruz) was coupled to protein A-Sepharose beads (Zymed Laboratories). Tissue lysates (350 μg of protein) were then incubated with the antibody coupled beads overnight at 4°C with rotation. Immunoprecipitated proteins for IRS-1 determination or equal amounts of protein for determination of Akt pSer473 (75 μg), PTP1B (10 μg), SHP2 (10 μg), IRS-1 (45μg), and Akt protein abundance (75 μg) were suspended in Laemmli sample buffer. Bound proteins were separated by SDS-PAGE, and electrotransfer of proteins from the gel to nitrocellulose membranes in transfer buffer (25 mM Tris, pH 8.3, 192 mM glycine, and 20% vol/vol methanol) was performed for 100 min at 100 V (constant). Blots were probed with anti-Akt pSer473 rabbit polyclonal, anti-PTP1B monoclonal mouse, anti-IRS-1 rabbit polyclonal, anti-Akt rabbit polyclonal or anti-phosphotyrosine PY99 monoclonal mouse antibodies. PTP1B blots were stripped using Restore (Pierce) and reprobed with anti-SHP2 monoclonal mouse to determine SHP2 protein abundance. Binding was viewed by enhanced chemiluminescence (Pierce) and quantified with Kodak 1D version 3.5 densitometry software (Eastman Kodak).

**IR content and autophosphorylation by ELISA.** The IR content and the level of autophosphorylation in skeletal muscle extracts was also quantified by an IR antibody “trap” activation assay using time-resolved fluorescence and Europium conjugated reagents (P. Hoyne and T. Adams, unpublished observations). Microtiter 96-well plates were coated with 83.7 anti-IR antibody [kindly provided by Ken Siddle, University of Cambridge (45)]. The plate wells were then blocked with 0.5% ovalbumin in 1× TBS. Aqueous samples of skeletal muscle extracts containing 100 μg of protein were applied to wells in triplicate, and then either Europium-labeled human recombinant insulin (1:4,000, a gift from Peter Hoyne, CSIRO Molecular and Health Technologies, Melbourne, Australia) or anti-phosphotyrosine (1:10,000, pY100 Perkin Elmer) antibody was added to each well and incubated overnight at 4°C. Finally, Europium enhancement solution was added and time-resolved fluorescence counted in a Wallac Victor fluorometer (Perkin Elmer).

**Statistical analysis.** Standards were included in all immunoblots, and interassay variation was accounted for by normalizing data to control samples. Data are presented as means ± SE. Differences were determined using one-way repeated-measures ANOVA for plasma and GIR measurements and two-way (insulin × exercise) repeated-measures ANOVA for skeletal muscle measurements, with Newman-Keuls post hoc analysis, where appropriate. Significance was accepted when P < 0.05.

**RESULTS**

**Hyperinsulinemic euglycemic clamp.** In the rested trial, infusion of insulin during the hyperinsulinemic-euglycemic clamp significantly increased (P < 0.05) plasma insulin to 422 ± 15 pM by 20 min, which remained constant for the duration of the clamp protocol. Identical insulin infusion rates were maintained for the subsequent clamps following the acute exercise bout and after 7 days exercise training. However, during the 90- to 120-min period of the clamp following the exercise training, plasma insulin levels were reduced on average by ~10% (P < 0.05; Table 1).

Fasting blood glucose levels were similar following the rested and exercise interventions. In each of the hyperinsulinemic-euglycemic clamps, blood glucose levels remained constant at 4.8 mM for 90–120 min of the clamp and were not significantly different between trials (Table 1). The GIR during the final 30 min of the clamp following an acute bout of exercise (6.2 ± 1.0 mg·kg−1·min−1) was not different when compared with the rested trial. However, following the exercise training, GIR was significantly increased ~30% (P < 0.05; Table 1) when compared with the rested trial, indicating an improvement in insulin sensitivity. Homeostasis model assessment (HOMA) values were significantly (P < 0.05; Table 1) reduced ~25% 24 h following acute exercise and ~35% following short-term training, consistent with improved insulin sensitivity (37).

**IR and IRS-1 protein expression and phosphorylation.** Protein expression of IR and IRS-1 24 h following either acute exercise or short-term training were similar to rested values (Table 2 and Fig. 1). Expression of these insulin-signaling proteins was also not affected by insulin infusion during the hyperinsulinemic-euglycemic clamp in any of the experimental trials (Table 2 and Fig. 1). Insulin infusion during the hyperinsulinemic-euglycemic clamp significantly increased IR and IRS-1 tyrosine phosphorylation (P < 0.05, main effect for insulin; Table 2 and Fig. 1). However, compared with the rested trial, acute exercise and training had no effect on basal or insulin-stimulated IR and IRS-1 tyrosine phosphorylation (Table 2 and Fig. 1).

**Akt phosphorylation and protein expression.** Akt protein expression levels were not affected by the acute exercise and training interventions, nor were they affected by insulin during the hyperinsulinemic-euglycemic clamp in each of the experimental trials (Table 2 and Fig. 1). Insulin infusion significantly increased Akt pSer473 (P < 0.05; Fig. 2) in all three trials. Furthermore, insulin-stimulated Akt pSer473 was ~50% higher (P < 0.05; Fig. 2) following the acute exercise and training interventions.

**SHP2 and PTP1B protein expression.** Insulin infusion had no effect on SHP2 protein abundance or subcellular localization in either the cytosolic or particulate fractions (Fig. 3, A and B, respectively). The exercise interventions resulted in significantly increased SHP2 protein abundance within the cytosolic fraction (P < 0.05 main effect for exercise; Fig. 3A) but not in the particulate fraction (Fig. 3B). Insulin and/or exercise had no effect on PTP1B protein abundance or subcellular localization (Table 2 and Fig. 1).

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Fasting plasma glucose and insulin levels, HOMA, and glucose, insulin and insulin-stimulated GIR values during the euglycemic-hyperinsulinemic clamps at rest (rested) and 24 h following a single exercise bout (acute) or short-term exercise training (trained)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rested</td>
<td>Acute</td>
</tr>
<tr>
<td>Fasting plasma glucose, mM</td>
<td>4.86±0.23</td>
</tr>
<tr>
<td>Fasting plasma insulin, pM</td>
<td>79.4±8.5</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>Plasma glucose, 90–120 min of clamp, mM</td>
<td>4.84±0.02</td>
</tr>
<tr>
<td>Plasma insulin, 90–120 min of clamp, pM</td>
<td>421.5±15.2</td>
</tr>
<tr>
<td>GIR, mg·kg⁻¹·min⁻¹</td>
<td>5.8±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8). HOMA, homeostasis model assessment; GIR, glucose infusion rate. 
*P < 0.05 vs. rested (one-way repeated-measures ANOVA).
the first (acute) exercise bout and following 7 days of exercise endurance training studies (21, 48, 61). A major finding was young, healthy, sedentary male cohort, consistent with similar exercise training (trained) 24 h following a single exercise bout (acute) or short-term hyperinsulinaemic-euglycemic clamp at rest (rested) and insulin signaling proteins in response to a Table 2.

strated the effectiveness of short-term exercise training in completion of the last exercise session. The results demonstrated the effectiveness of short-term exercise training in enhancing insulin-mediated glucose disposal by ∼30%, in a young, healthy, sedentary male cohort, consistent with similar endurance training studies (21, 48, 61). A major finding was the ∼50% increase in insulin-stimulated Akt pSer473 24 h after the first (acute) exercise bout and following 7 days of exercise training. No further changes were evident in the upstream components of the insulin signaling pathway, including tyrosine phosphorylation of IR or IRS-1. A further finding of the present study was increased protein abundance of SHP2, a putative positive regulator of insulin signaling in the cytosolic fraction. These data provide evidence that the actions of physical activity on insulin signaling may extend beyond alterations in kinase activity to the regulation of phosphatases such as SHP2 that are implicated in cell signaling control.

There is strong evidence of increased Akt pSer473 in human skeletal muscle immediately after endurance exercise (14, 22, 42, 57). Studies reporting conflicting findings in human skeletal muscle could possibly be explained by delayed time of postexercise biopsy (~9 min) (51) or insufficient sample size (n = 3 subjects for Akt activity) (56). However, the activation of Akt Thr308 immediately postexercise in human skeletal muscle is less certain (14, 42). Nevertheless, the present study found basal Akt pSer473 was not higher 24 h following a single exercise bout or short-term training, in agreement with similar findings in rodents (10). Regardless of the impact of exercise on basal Akt pSer473, exercise training in rodents enhances insulin-stimulated Akt pSer473 for up to 48 h following exercise cessation (10, 34). Furthermore, this is the first study in human skeletal muscle to investigate IR, IRS-1, and Akt signaling 24 h following a single bout of exercise. The present study extends the previous findings in rodents (10, 34) to demonstrate a robust enhancement in the insulin-mediated activation of Akt pSer473 within 24 h following both a single acute exercise bout and short-term training. Also, the increased insulin-stimulated Akt pSer473 observed in the present study is likely due to the residual effects of the prior bout of exercise, since, compared with the single exercise bout, there was no further increase following short-term training.

The present study also observed dissociation between increased insulin-stimulated Akt pSer473 and whole body insulin sensitivity 24 h after acute exercise and short-term training. GIR and Akt pSer473 are both insulin-mediated effects; however, insulin-stimulated Akt pSer473 improved following acute exercise despite no change in GIR. It is difficult to draw too

### Table 2. Protein expression and tyrosine phosphorylation of insulin signaling proteins in response to a hyperinsulinaemic-euglycemic clamp at rest (rested) and 24 h following a single exercise bout (acute) or short-term exercise training (trained)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Rested</th>
<th>Acute</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin receptor protein</td>
<td>5.74±0.53</td>
<td>5.32±0.56</td>
<td>5.43±0.70</td>
</tr>
<tr>
<td>Insulin</td>
<td>5.23±0.55</td>
<td>5.79±0.59</td>
<td>6.36±0.62</td>
</tr>
<tr>
<td>pTyr, insulin receptor</td>
<td>0.60±0.04</td>
<td>0.52±0.05</td>
<td>0.53±0.08</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.19±0.15†</td>
<td>1.09±0.10†</td>
<td>1.03±0.07*</td>
</tr>
<tr>
<td>IRS-1 protein</td>
<td>2.22±0.70</td>
<td>1.54±0.43</td>
<td>1.18±0.16</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.78±0.63</td>
<td>1.50±0.44</td>
<td>1.53±0.47</td>
</tr>
<tr>
<td>pTyr-IRS-1</td>
<td>0.10±0.04</td>
<td>0.13±0.07</td>
<td>0.06±0.03</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.22±0.08*</td>
<td>0.19±0.08*</td>
<td>0.12±0.04*</td>
</tr>
<tr>
<td>Akt protein</td>
<td>5.54±2.12</td>
<td>8.40±2.36</td>
<td>9.06±4.45</td>
</tr>
<tr>
<td>Insulin</td>
<td>7.41±2.22</td>
<td>7.20±2.45</td>
<td>7.54±2.28</td>
</tr>
<tr>
<td>PTP1B cytosol protein</td>
<td>6.118±1.683</td>
<td>5.550±1.670</td>
<td>4.311±1.785</td>
</tr>
<tr>
<td>Insulin</td>
<td>5.177±1.827</td>
<td>5.004±1.058</td>
<td>3.560±0.964</td>
</tr>
<tr>
<td>PTP1B particulate protein</td>
<td>22.607±2.267</td>
<td>22.967±2.807</td>
<td>22.492±2.546</td>
</tr>
<tr>
<td>Insulin</td>
<td>20.083±2.578</td>
<td>23.041±2.828</td>
<td>24.218±1.140</td>
</tr>
</tbody>
</table>

Values are expressed as arbitrary densitometry units (means ± SE); n = 8 for Akt protein, insulin receptor protein, and pTyr; n = 7 for IRS-1 protein and pTyr due to lack of sufficient sample. Insulin, insulin-stimulated; IRS-1, insulin receptor substrate-1; pTyr, tyrosine phosphorylation. *P < 0.05 insulin (main) effect (two-way repeated-measures ANOVA).

**DISCUSSION**

Regular physical activity enhances insulin sensitivity. The present study investigated the effects of a single exercise bout (acute) and 7 days of repeated cycling training on insulin sensitivity and skeletal muscle insulin signaling 24 h after the completion of the last exercise session. The results demonstrated the effectiveness of short-term exercise training in enhancing insulin-mediated glucose disposal by ~30%, in a young, healthy, sedentary male cohort, consistent with similar endurance training studies (21, 48, 61). A major finding was the ~50% increase in insulin-stimulated Akt pSer473 24 h after the first (acute) exercise bout and following 7 days of exercise...
many conclusions between measurements of whole body insulin sensitivity and skeletal muscle insulin signaling. In part, this uncoupling may reflect the involvement of Akt in pathways other than glucose uptake, such as insulin-stimulated glycogen or protein synthesis (28, 52). Furthermore, although pSer473 is important for Akt phosphorylation (44) and necessary for the full activation of Akt kinase activity (44), increased Akt pSer473 may not be sufficient for increased Akt activity (29, 47). Thus it is possible that increased insulin-stimulated pSer473 Akt in the present study may not correlate to Akt activity. The absence of a direct relationship between whole body insulin sensitivity and skeletal muscle Akt pSer473 following acute exercise is an interesting finding that warrants further investigation.

Future studies may be able to address the signaling role(s) of increased insulin-stimulated pSer473 Akt following exercise by examining the insulin-stimulated phosphorylation of the various Akt isoforms following exercise. Three mammalian isoforms of Akt have been identified to date: Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ) (36). Akt1 is 81 and 83% homologous to Akt2 and Akt3, respectively (36). Akt2 appears to play more of a role in insulin-stimulated GLUT4 translocation and glucose uptake (5, 20, 26, 43), whereas Akt1 and Akt2 both appear involved in glycogen synthesis (26). The polyclonal antibody used in this study for the quantification of the activating pSer473 residue on Akt1 may detect the equivalent sites on Akt2 (pSer472) and Akt3 (pSer472) (36), although direct evidence is lacking. Supporting this conjecture are recent data in human skeletal muscle demonstrating that siRNA-based gene silencing of Akt2, rather than Akt1, abolished insulin-mediated phosphorylation using a Ser473 Akt1-specific antibody (5). Several studies have also demonstrated impaired activation of Akt2 in insulin-resistant states (7, 18). Therefore, future investigation is required to determine whether increased insulin-stimulated Akt pSer473 following acute exercise and short-term training is localized to the Akt2 isoform, implicated in the regulation of glucose transport.

No previous study has examined the impact of physical activity on the cellular abundance and localization of the major PTPases in human skeletal muscle. The present study found that acute exercise and short-term training increased cytosolic SHP2 protein expression (P < 0.05; main effect for exercise; Fig. 3A). Within skeletal muscle SHP2 accounts for the large majority of cytosolic PTPase activity (2). Unlike the majority of the phosphatase family of proteins, SHP2 is implicated in the positive regulation of insulin-stimulated glucose transport, glycogen, and protein synthesis (54). The overexpression of SHP2 in rat fibroblasts enhances insulin-stimulated IRS-1 tyrosine phosphorylation and the associated PI3-kinase activity, whereas inhibition of SHP2 has the opposite effect (54). Transgenic mice with impaired SHP2 not only display diminished insulin-stimulated glucose transport but also lower glycogen synthase activity and MAP kinase phosphorylation, suggesting multiple regulatory roles for SHP2 in skeletal muscle (35). Based on the data from the present study, it is not possible to draw conclusions with respect to the possible roles of the increased cytosolic SHP2. One of the main regulatory mechanisms for SHP2 on insulin signaling involves increased IRS-1 tyrosine phosphorylation (35, 54), although the present study observed no changes in IRS-1 tyrosine phosphorylation following acute exercise or short-term training. Also, our group has previously demonstrated that acute exercise does not increase the localization of IRS-1 and IRS-2 to the cytosolic fraction (57), thus ruling out greater colocalization of SHP2 to its major substrates. However, it is possible that SHP2 is involved in signaling and activation of Akt independently of IRS-1, since SHP2 is also involved in Src phosphorylation (55) that can subsequently tyrosine phosphorylate and activate Akt (23). Nevertheless, the role for increased cytosolic SHP2 following acute exercise and short-term training is an important question that remains to be answered.

The actions of exercise did not extend to PTP1B, a potent negative regulator of the insulin signaling pathway (40). Few studies have demonstrated in vivo regulation in either PTP1B abundance or activity, although chronic hyperinsulinemia in rodents increased localization of PTP1B with the IR (53) and weight loss in obese humans is associated with reduced adipose tissue PTP1B protein abundance (1), consistent with increased

![Fig. 3. Protein expression of SHP2 in a cytosol (A) and particulate (B) fraction in response to a hyperinsulinemic-euglycemic clamp at rest (rested) and 24 h following a single exercise bout (acute) or short-term exercise training (trained). Western blots are representative from one subject. Values are means ± SE (n = 8). §P < 0.05 exercise (main) effect (two-way repeated-measures ANOVA).](http://jap.physiology.org/images/jap10021002/jap10021002.jpg)
PTP1B activity in insulin-resistant rodent models (13). However, the present study failed to demonstrate significant regulation of PTP1B cellular content and localization by either acute insulin infusion or exercise. The present study found that acute exercise and short-term training did not alter the insulin-stimulated increase in either protein abundance or phosphorylation state of IR and IRS-1, despite improvements in whole body insulin action. A significant increase in insulin-stimulated in vitro IR autophosphorylation and in vivo total phosphoryrosine PI3-kinase activity has previously been reported in human muscle following similar short-term endurance training (21, 61), although the improvements in PI3-kinase activity are not apparent in middle-aged men (48). Importantly, these previous studies used insulin levels approximately three times greater than those used in the current study (21, 61), making comparisons difficult. Thus, under conditions of modest hyperinsulinemia, 24 h after a preceding exercise bout or with exercise training, no changes were evident in the tyrosine phosphorylation of proximal steps in the insulin-signaling cascade.

In the present study, GIR was not significantly increased 24 h following acute exercise. This is in contrast to previous studies (12, 38, 51, 60) utilizing the euglycemic, hyperinsulinemic clamp procedure. However, comparisons between studies are problematic, since previous studies have utilized either much higher levels of insulin infusion in conjunction with glucose tracer methodologies (38), subjects with impaired glucose tolerance (12), or the clamp procedure within a few hours of exercise cessation (51, 60). Of interest was the reduced HOMA values following acute exercise (~25%) and short-term exercise training (~35%), consistent with improved insulin sensitivity (37) and likely due to the reduced fasting insulin levels (Table 1). Caution must be exercised in applying HOMA analysis to small subject groups due to its high degree of imprecision for estimating insulin resistance (coefficient of variation = 31%), particularly from a single fasting plasma insulin analysis (37). However, our finding of ~30% increased GIR following short-term training, consistent with previous studies (21, 48, 61), suggests that the effects of cumulative bouts of exercise on insulin sensitivity are much greater than a single exercise bout.

In the present study, plasma insulin concentration during the clamp was significantly reduced following short-term training, despite insulin infusion rates remaining constant. This suggests increased insulin clearance following short-term training, although the body of evidence for this is equivocal (6, 21, 39, 48, 51, 58). Similar short-term training studies, albeit with much higher insulin infusion levels, do not support improved insulin clearance (21, 48). However, endurance training increases hepatic insulin clearance in rodents (58), whereas whole body insulin clearance in humans is increased for up to 18 h following acute exercise (6, 39, 51).

In summary, this study found that insulin-stimulated Akt pSer\textsuperscript{473} is increased 24 h following acute exercise and short-term training in human skeletal muscle and appears largely due to residual effects of the prior bout of exercise. There was no effect of acute exercise and short-term training on the upstream components of the insulin-signaling pathway such as IR or IRS-1 tyrosine phosphorylation. Also, there was dissociation between improved whole body insulin sensitivity and insulin-stimulated Akt pSer\textsuperscript{473} following acute exercise and short-term training. The present study also observed increased abundance of SHP2 protein in the cytosolic fraction of skeletal muscle 24 h after acute exercise and short-term training. These novel findings may contribute to the improvement in insulin sensitivity that accompanies regular physical activity. Further studies are required to determine which Akt isoform is predominately influenced and the exact role of SHP2 in regulating in vivo insulin signaling.

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