The effect of exercise and insulin on AS160 phosphorylation and 14-3-3 binding capacity in human skeletal muscle

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Howlett KF, Mathews A, Garnham A, Sakamoto K. The effect of exercise and insulin on AS160 phosphorylation and 14-3-3 binding capacity in human skeletal muscle. Am J Physiol Endocrinol Metab 294: E401–E407, 2008. First published November 27, 2007; doi:10.1152/ajpendo.00542.2007.—AS160 is an Akt substrate of 160 kDa implicated in the regulation of both insulin- and contraction-mediated GLUT4 translocation and glucose uptake. The effects of aerobic exercise and subsequent insulin stimulation on AS160 phosphorylation and the binding capacity of 14-3-3, a novel protein involved in the dissociation of AS160 from GLUT4 vesicles, in human skeletal muscle are unknown. Hyperinsulinemic-euglycemic clamps were performed on seven men at rest and immediately and 3 h after a single bout of cycling exercise. Skeletal muscle biopsies were taken before and after the clamps. The insulin sensitivity index calculated during the final 30 min of the clamp was 8.0 ± 0.8 for the rest, postexercise, and 3-h postexercise trials, respectively. AS160 phosphorylation increased immediately after exercise and remained elevated 3 h after exercise. In contrast, the 14-3-3 binding capacity of AS160 and phosphorylation of Akt and AMP-activated protein kinase were only increased immediately after exercise. Insulin increased AS160 phosphorylation and 14-3-3 binding capacity and insulin receptor substrate-1 and Akt phosphorylation, but the response to insulin was not enhanced by prior exercise. In conclusion, the 14-3-3 binding capacity of AS160 is increased immediately after acute exercise in human skeletal muscle, but this is not maintained 3 h after exercise completion despite sustained AS160 phosphorylation. Insulin increases AS160 phosphorylation and 14-3-3 binding capacity, but prior exercise does not appear to enhance the response to insulin.

glucose transport; type 2 diabetes

THE GLUCOSE TRANSPORTER GLUT4/SLC2A4 plays an important role in glucose homeostasis by regulating glucose uptake in skeletal muscle and adipose tissue (11). Insulin regulates GLUT4 translocation from an intracellular location to the cell surface via a well-described pathway involving phosphatidylinositol 3-kinase and Akt/PKB. However, the specific link between the insulin signaling pathway downstream of Akt and GLUT4 translocation is less well characterized. Recent interest has focused on an Akt substrate of 160 kDa (AS160; also known as Tbc1d4), a Rab GTPase-activating protein (GAP) (12, 27). The present hypothesis for the role of AS160 in mediating insulin-stimulated GLUT4 translocation is that in the basal state AS160 is bound to GLUT4 storage vesicles (GSV), in part via a direct interaction with the insulin responsive aminopeptidase (22), which retains GLUT4 to intracellular compartments. In response to insulin, and in an Akt-dependent manner, AS160 is phosphorylated, subsequently leading to inactivation of the Rab-GAP activity of AS160 and relieving the inhibitory effect on GLUT4 translocation to the cell surface.

Exercise or skeletal muscle contraction also increases glucose uptake by GLUT4 translocation to the cell surface. The signaling pathway(s) mediated by exercise or contraction is generally thought to be distinct from that of insulin, although common points of convergence may exist between the signaling pathways (25). Given that AS160 is phosphorylated by exercise in humans (4, 28, 32) and in rodents after in vivo (1) and in vitro muscle contraction (2, 16, 17), AS160 has been proposed to be a point of convergence. In further support of this, AS160 phosphorylation is increased in rodent skeletal muscle in an additive manner in response to insulin stimulation and in vitro muscle contraction (17) or 4 h after swimming exercise (1). In contrast, in skeletal muscle of humans with type 2 diabetes, insulin-stimulated AS160 phosphorylation was not enhanced during low-intensity exercise compared with insulin stimulation alone (13). These results could be due to differences in species or timing of the insulin treatment, or, alternatively, exercise-induced phosphorylation of AS160 may be attenuated in skeletal muscle of humans with Type 2 diabetes (28). An aim of this study was therefore to further examine the effect of exercise and subsequent insulin stimulation on the regulation of AS160 in skeletal muscle of healthy humans.

The function of AS160 in insulin-mediated GLUT4 translocation is linked to its interaction with a novel binding partner, 14-3-3 (8, 23). It has been reported that 14-3-3 interacts with AS160 in an insulin- and Akt-dependent manner via the Akt Thr642 phosphorylation site (23), and potentially this interaction may lead to dissociation of AS160 from the GSV. In support of a physiological role, the 14-3-3 binding capacity of AS160 is increased by insulin in human skeletal muscle (10). In contrast, a single acute bout of resistance exercise impairs AS160 phosphorylation and its 14-3-3 binding capacity, an effect likely mediated through a decrease in Akt phosphorylation (13). These results could be due to differences in species or timing of the insulin treatment, or, alternatively, exercise-induced phosphorylation of AS160 may be attenuated in skeletal muscle of humans with Type 2 diabetes (28). An aim of this study was therefore to further examine the effect of exercise and subsequent insulin stimulation on the regulation of AS160 in skeletal muscle of healthy humans.

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performed at rest and immediately and 3 h after a single acute bout of aerobic exercise in healthy humans. We hypothesized that acute aerobic exercise would increase AS160 phosphorylation and 14-3-3 binding capacity in human skeletal muscle and, in the period after exercise, that the insulin-stimulated increase in AS160 phosphorylation and 14-3-3 binding capacity would be further enhanced compared with that shown with insulin stimulation alone.

**METHODS**

**Subjects.** Seven untrained men (21.9 ± 0.6 yr, 79.5 ± 4.0 kg, 184.8 ± 3.5 cm; means ± SE) volunteered as subjects for the experiment. Experimental procedures and possible risks of the study were explained verbally and in writing. All subjects gave their informed, written consent, and the experiment was approved by Deakin University Human Research Ethics Committee. Before the experiment, subjects performed an incremental workload test to exhaustion on an electromagnetically braked cycle ergometer (Lode, Groningen, The Netherlands) to determine their peak pulmonary oxygen uptake (VO2peak) as previously described (9). VO2peak was 3.64 ± 0.28 l/min.

**Experimental protocol.** For each experimental trial, subjects presented to the laboratory in the morning after an overnight fast (10–12 h). On the day before each trial, subjects consumed a standardized diet (~14,500 kJ, ~90% carbohydrate). In the 24 h before each trial, subjects avoided the consumption of tobacco, alcohol, and caffeine. For 48 h before the first randomized trial and then for the entire duration of the research study, subjects refrained from any physical activity other than that associated with the experimental trials.

The experiment consisted of three randomized trials in which a hyperinsulinemic-euglycemic clamp was performed either 1) at rest (Rest trial), 2) immediately after a single bout of aerobic exercise (Post-Ex trial), or 3) 3 h after a single bout of aerobic exercise (3 h Post-Ex trial) (Fig. 1). The exercise bout consisted of 60 min of cycle ergometer exercise at an intensity of ~60% VO2peak. In the 3 h Post-Ex trial, subjects remained resting in a semi-supine position for the 3 h after exercise and consumed only water.

The hyperinsulinemic-euglycemic clamps were performed as previously described (9). The glucose infusion rate during the last 30 min of the clamp was used as a measure of glucose disposal rate (M; in mg·kg⁻¹·min⁻¹). The insulin sensitivity index (M-to-I ratio) was calculated by dividing M by the mean insulin concentration (I; in nM/l) during the same period of the clamp. M/I represents the amount of glucose disposed per unit of plasma insulin [mg·kg⁻¹·min⁻¹·(nM/l)⁻¹ × 100]. Blood was sampled at rest and during exercise and the clamp for analysis of blood glucose and lactate (EMI 105 Radiometer, Copenhagen, Denmark) and plasma insulin (human insulin-specific RIA kit; Linco Research, St. Charles, MO). Muscle samples were obtained from the vastus lateralis, by the percutaneous needle biopsy technique modified for suction (6) before (without insulin) and at the completion (with insulin) of each clamp.

**Preparation of skeletal muscle lysate.** Skeletal muscle was homogenized (Kinematica Polytron, Brinkmann, CT) in ice-cold buffer [20 mM Tris·HCl, 5 mM EDTA, 10 mM Na2PO4·7H2O, 100 mM NaF, 2 mM Na3VO4, 1% Nonidet P-40, and complete protease inhibitor cocktail (Roche, Lewes, Sussex, UK)] and incubated for 30 min at 4°C. Homogenates were spun at 13,000 g for 30 min to remove insoluble material. Supernatants were collected, and aliquots were snap frozen and stored at −80°C for later analysis. Total protein concentrations were determined by the Bradford method using BSA as standard.

**Antibodies.** Phosphospecific antibodies Akt [Ser73 (no. 9271) and Thr66 (no. 9208), phosho-Ser/Thr Akt substrate antibody (no. 9611)], AMP-activated protein kinase (AMPK)−α2 (Thr172; no. 2531), and acetyl-CoA carboxylase (ACC)−β (Ser211; no. 3661) were from Cell Signaling Technology (Beverly, MA). Total insulin receptor substrate-1 (IRS-1; no. 06-248) and AS160 (Rab-GAP; no. 07-741) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The total AS160 antibody for immunoprecipitation was raised in sheep against the peptide CHPTNDKAKAGNKP (Cys + mouse residue 1295–1307), and total AMPK−α2 antibodies were kindly donated by Professor Graham Hardie (University of Dundee). ExtraAvidin peroxidase conjugate used to detect ACC was from Sigma. Secondary antibodies coupled to horseradish peroxidase were from Pierce (Rockford, IL). Anti-digoxigenin (DIG) coupled to horseradish peroxidase was from Roche.

**Immunoblotting.** Skeletal muscle lyses (40–60 µg) were heated in SDS sample buffer, separated by gel electrophoresis using precast SDS-polyacrylamide Bis-Tris gels (Invitrogen, Paisley, UK), and transferred to nitrocellulose membranes. Membranes were incubated for 1 h in blocking buffer [50 mM Tris·HCl, pH 7.5, 0.15 M NaCl, and 0.5% Tween 20 (TBST)] containing 10% milk or 5% BSA. Membranes were then incubated overnight at 4°C with 1 µg/ml for the sheep or 1,000-fold dilution for commercial antibodies in TBST containing either 5% milk or 5% BSA. Detection of proteins was performed using horseradish peroxidase-conjugated secondary antibody in 5% milk in TBST for 60 min. Antibody binding was viewed by enhanced chemiluminescence. Film was scanned by ChemiGenius Bio-Imaging system (SynGene, Cambridge, UK), and bands were identified and quantified with Gene Tools analysis software (Syngene).

**Immunoprecipitation.** Skeletal muscle lyses (300–500 µg) were incubated at 4°C for 1 h on a shaking platform with 5 µl of protein G-Sepharose (Amersham Biosciences, Little Chalfont, UK) coupled to 3–4 µg AS160 or IRS-1 antibody. Immunoprecipitates were washed with buffer A [50 mM Tris·HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, 1% (mass) Triton X-100, 1 mM Na2VO4, 50 mM NaF, 5 mM Na3PO4·7H2O, 0.27 mM succrose, 0.5 M NaCl] and buffer B (50 mM Tris·HCl, pH 7.5, 0.1 mM EGTA). Samples were heated in SDS sample buffer, separated by gel electrophoresis, and immunoblotted as described above.

**DIG-14-3-3 overlays.** For DIG-14-3-3 overlays, AS160 was immunoprecipitated from skeletal muscle lysate as described above.

After separation of proteins by gel electrophoresis, the DIG-labeled 14-3-3 overlays. After separation of proteins by gel electrophoresis, the DIG-labeled 14-3-3 overlays were incubated with the DIG-14-3-3 overlays. For DIG-14-3-3 overlays, AS160 was immunoprecipitated from skeletal muscle lysate as described above.

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Exercise. Exercise intensity and cardiorespiratory measures were similar between the exercise trials (Post-Ex, 3 h Post-Ex). For both trials, the average exercise intensity, respiratory exchange ratio, ventilation, and heart rate results were 59.2 ± 0.22% VO2peak, 1.0 ± 0.0 arbitrary units, 48.9 ± 1.9 l/min, and 152 ± 7 beats/min, respectively. Plasma glucose levels did not change during the 60 min of exercise and were similar between trials, with plasma glucose levels in each trial averaging 4.6 ± 0.1 (Post-Ex) and 4.8 ± 0.1 mmol/l (3 h Post-Ex). Plasma lactate levels were similar between trials before exercise (Post-Ex: 1.4 ± 0.2 mmol/l, 3 h Post-Ex: 1.5 ± 0.4 mmol/l). In response to exercise, lactate levels increased significantly at 10 min (Post-Ex: 4.7 ± 0.3 mmol/l, 3 h Post-Ex: 5.1 ± 0.4 mmol/l) and remained elevated (P < 0.05) for the duration of exercise (60 min Post-Ex: 3.4 ± 0.4 mmol/l, 3 h Post-Ex: 3.7 ± 0.4 mmol/l). Plasma insulin levels decreased (P < 0.05, time effect) during exercise from basal (Post-Ex: 60.6 ± 6.9 pmol/l, 3 h Post-Ex: 73.4 ± 14.9 pmol/l) to 60 min (Post-Ex: 42.8 ± 2.9 pmol/l, 3 h Post-Ex: 41.7 ± 5.9 pmol/l).

Hyperinsulinemic-euglycemic clamp. Insulin infusion during the clamp increased (P < 0.05, main effect) plasma insulin from basal (Rest: 68 ± 9 pmol/l, Post-Ex: 54 ± 6 pmol/l, 3 h Post-Ex: 57 ± 14 pmol/l) to high physiological levels, with the average insulin concentration during the final 30 min of the clamp similar in all trials (Rest: 720 ± 42 pmol/l, Post-Ex: 668 ± 45 pmol/l, 3 h Post-Ex: 629 ± 17 pmol/l). In each trial, exogenous glucose was variably infused such that blood glucose levels remained at ~5 mmol/l for the duration of the clamp (Rest: 4.8 ± 0.1 mmol/l, Post-Ex: 4.8 ± 0.1 mmol/l, 3 h Post-Ex: 4.9 ± 0.0 mmol/l, average final 30 min of clamp). Glucose infusion rates during the final 30 min of the clamp were 9.3 ± 0.5, 9.8 ± 0.3, and 9.3 ± 0.7 mg·kg⁻¹·min⁻¹ for the Rest, Post-Ex, and 3 h Post-Ex trials, respectively. The insulin sensitivity index was also calculated during the final 30 min of the clamp, taking into consideration the plasma insulin levels in each trial. The insulin sensitivity indexes calculated during the final 30 min of the clamp were 8.0 ± 0.8, 9.1 ± 0.5, and 9.2 ± 0.8 for the Rest, Post-Ex, and 3 h Post-Ex trials, respectively. Blood lactate levels were elevated (P < 0.05) at the commencement of the clamp in the Post-Ex group (1.7 ± 0.4 mmol/l) but not in the Rest (1.4 ± 0.2 mmol/l) or 3 h Post-Ex groups (1.5 ± 0.3 mmol/l). In the Post-Ex trial, blood lactate levels returned within 30 min to levels similar to those in the other trials. The average blood lactate levels measured during the final 30 min of the clamp were 1.5 ± 0.1, 1.2 ± 0.1, and 1.2 ± 0.1 mmol/l for the Rest, Post-Ex, and 3 h Post-Ex trials, respectively.

Insulin signaling proteins. A single acute bout of cycling exercise significantly increased AS160 phosphorylation at phospho-Akt substrate motifs by ~1.8 fold, and levels remained elevated for at least 3 h after exercise (Figs. 2A and 3). Insulin significantly increased phosphorylation of AS160 by two- to threefold from basal levels (P < 0.05, main effect), and this response was similar immediately and 3 h after exercise. Total AS160 protein levels were not different in response to exercise and/or insulin (mean data not shown; representative blot in Fig. 2B).

AS160 is a downstream target of Akt; immediately after a single exercise bout, phosphorylation of Akt Ser473 (Figs. 2A and 4) increased significantly but not Akt Thr308 (without insulin), Rest = 1.23 ± 0.26, Post-Ex = 1.85 ± 0.28, 3 h Post-Ex = 1.33 ± 0.13 arbitrary units; representative blot in Fig. 2A). Insulin stimulation resulted in an increase (P < 0.05, main effect) in Akt Ser473 (Figs. 2A and 4) and Thr308 (with insulin, Rest = 4.21 ± 0.53, Post-Ex = 4.53 ± 0.52, and 3 h Post-Ex = 4.21 ± 0.40 arbitrary units; representative blot in Fig. 2A) that was similar in all trials. Total Akt protein levels were similar in response to exercise and/or insulin (mean data not shown; representative blot in Fig. 2B). Basal IRS-1 tyrosine phosphorylation was not altered either immediately or 3 h after exercise (Figs. 2A and 4). Insulin infusion resulted in a similar increase (P < 0.05, main effect) in IRS-1 tyrosine phosphorylation in all trials. Total IRS-1 protein levels were not different in response to exercise and/or insulin (mean data not shown; representative blot in Fig. 2B).

DIG 14-3-3 overlays. Similar to results for AS160 phosphorylation, the 14-3-3 binding capacity of AS160 was significantly increased immediately after a single bout of exercise; however, in contrast to AS160 phosphorylation, this effect was not maintained 3 h after exercise (Figs. 2A and 3). In response to

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**Fig. 2.** Phosphorylation (A) and total protein (B) of insulin signaling proteins in response to a hyperinsulinemic-euglycemic clamp in the 3 trials (Rest, Post-Ex, and 3 h Post-Ex). Data are representative blots. p, Phosphorylation; IRS-1, insulin receptor substrate-1; AS160, Akt substrate of 160 kDa; AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase.

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<td>Akt pSer473</td>
<td>Akt pThr308</td>
<td>p-AS160</td>
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<td>14-3-3 overlay</td>
<td>AMPK pThr172</td>
<td>ACC pSer21</td>
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**B**

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AS160 phosphorylation, detected by a phospho-Akt substrate antibody, was increased immediately after exercise and remained elevated for 3 h after the completion of exercise. These findings provide further support that acute aerobic exercise increases AS160 phosphorylation in a time-dependent manner in human skeletal muscle (28, 32) and that AS160 phosphorylation remains elevated in skeletal muscle for a prolonged period after exercise (28). Sustained AS160 phosphorylation 4 h after the completion of exercise has also been shown in rodent skeletal muscle and may explain the small increase in muscle glucose transport observed in that study (1). The phosphorylation of AS160 and GLUT4 translocation has been linked to the interaction with a novel binding partner, 14-3-3 (23). Our group (10) has recently demonstrated that resistance exercise reduces the binding capacity between these signaling proteins, although it appears that exercise mode differentially regulates this interaction because the 14-3-3 binding capacity of AS160 was significantly increased by acute aerobic exercise in the present study. However, unlike AS160 phosphorylation, the 14-3-3 binding capacity of AS160 was not maintained 3 h after exercise. The interplay between AS160 and 14-3-3 is complex, and, although insulin-mediated 14-3-3 binding to AS160 is mainly mediated through phosphorylation of Thr642, the phosphorylation state of other sites, such as Ser341 and Ser318 (which cannot be efficiently detected by the phospho-Akt substrate antibody), may influence this interaction.

**Discussion**

AS160 has recently emerged as a key signaling component in muscle glucose transport and uptake in response to both exercise and insulin and may be a common point of convergence between the signaling pathways regulating GLUT4 translocation. To further examine the effect of exercise and subsequent insulin stimulation on the regulation of AS160 and its binding capacity with 14-3-3 in skeletal muscle, hyperinsulinemic-euglycemic clamps were performed at rest and immediately and 3 h after an acute bout of aerobic exercise in humans.

**Figure 3.** Phosphorylation of AS160 (top) and 14-3-3 binding capacity of AS160 (bottom) in response to a hyperinsulinemic-euglycemic clamp in the 3 trials (Rest, Post-Ex, and 3 h Post-Ex). Values are means ± SE (n = 7 men). Open bars denote experiments without insulin (Ins), whereas solid bars denote those with insulin. *P < 0.05, significant difference between Rest vs. exercise (Post-Ex, 3 h Post-Ex) under conditions without insulin stimulation. **P < 0.05, significant effect of insulin stimulation within groups (Rest, Post-Ex, 3 h Post-Ex). †P < 0.05, significant (main) effect of insulin stimulation.

**Figure 4.** Insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation (top) and Akt Ser473 phosphorylation (bottom) in response to a hyperinsulinemic-euglycemic clamp in the 3 trials (Rest, Post-Ex, and 3 h Post-Ex). Values are means ± SE (n = 7 men). Open bars denote experiments without insulin, whereas solid bars denote those with insulin. *P < 0.05, significant differences between Rest vs. exercise (Post-Ex, 3 h Post-Ex) under conditions without insulin stimulation. †P < 0.05, significant (main) effect of insulin stimulation.

**Figures:**

- **Figure 3.** Phosphorylation of AS160 (top) and 14-3-3 binding capacity of AS160 (bottom) in response to a hyperinsulinemic-euglycemic clamp in the 3 trials (Rest, Post-Ex, and 3 h Post-Ex). Values are means ± SE (n = 7 men). Open bars denote experiments without insulin (Ins), whereas solid bars denote those with insulin. *P < 0.05, significant difference between Rest vs. exercise (Post-Ex, 3 h Post-Ex) under conditions without insulin stimulation. **P < 0.05, significant effect of insulin stimulation within groups (Rest, Post-Ex, 3 h Post-Ex). †P < 0.05, significant (main) effect of insulin stimulation.

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interaction (8), although this is equivocal (23). It is also possible that the interaction between these proteins is dynamic and may only need to occur transiently to allow for 14-3-3 to assist in inactivation of AS160 Rab-GAP activity and/or dissociation from the GSV. It should also be noted that, although the 14-3-3 overlay assay is an established and valuable method to determine the binding capacity of 14-3-3 to target proteins that contain specific phospho-serine and -threonine motifs (8, 23), 14-3-3 binding sites in many proteins do not necessarily confirm to these optimal motifs, presumably because other structural features contribute to the interactions (18). In future studies, it will be important to detect and quantify 14-3-3 association from the GSV. It should also be noted that, although species differences or the experimental model and design. It is always observed (30, 32, 33). It is difficult, if not impossible, to determine the relative importance of these signaling pathways for AS160 regulation. However, as previously discussed for 14-3-3, it is possible that sustained activation of AS160 may occur in response to a brief transient interaction with Akt and/or AMPK. Alternatively, there may be timing differences associated with potential regulatory processes that act to dephosphorylate or inactivate AS160 and/or associated signaling pathways.

In response to insulin stimulation, and as previously measured in human (10, 13) and rodent skeletal muscle (17, 31), AS160 phosphorylation increased two- to threefold above basal levels under resting conditions. The binding capacity of AS160 and 14-3-3 and the phosphorylation of upstream insulin signaling proteins including IRS-1 and Akt were also increased with insulin stimulation alone. However, in contrast to our hypothesis, prior aerobic exercise did not enhance insulin-stimulated AS160 phosphorylation or other components in the insulin-signaling pathway compared with insulin stimulation alone. These results for AS160 are similar to a previous study that was performed in humans with Type 2 diabetes (13) but contrast with results from rodent skeletal muscle with combined treatment of insulin and electrically induced muscle contraction in vitro (17) or after swimming exercise (1). The reason for the contrasting results is unknown but could be due to species differences or the experimental model and design. It is possible that, in human skeletal muscle, prior exercise may inhibit or suppress the effect of subsequent insulin stimulation on AS160 phosphorylation. However, interpretation of the results is complicated as the phospho-Akt substrate antibody is designed to detect a number of putative Akt-regulated phosphorylation sites on AS160 that contain the RXRXXS/T (X = any amino acid) motif. Recent studies have shown that the phospho-Akt substrate antibody utilized in this study primarily detects phospho-Thr\(^{642}\) (8, 23), although different batches of antibody may display differential sensitivity to some extent. At least eight specific residues on AS160 have now been identified as being phosphorylated by signals from the phosphatidylinositol 3-kinase, PKC/Erk/RSK, and LKB1/AMPK pathways (8, 29). Insulin-like growth factor-1 robustly stimulated all eight phosphorylation sites, whereas in contrast 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, a pharmacological activator of AMPK, can increase AS160 phosphorylation in rodent muscle (17). Studies in transgenic animal models also suggest that AS160 phosphorylation is regulated, at least in part, through AMPK (17, 31) and in particular through AMPK complexes containing the α2-subunit (32). In response to acute moderate intensity exercise in humans, AMPK is activated in skeletal muscle (7, 35). Under similar exercise conditions in human skeletal muscle, Akt phosphorylation and/or activity is also increased (4, 9, 26, 34), although this response is not always observed (30, 32, 33). It is difficult, if not impossible, in human studies to definitively determine whether these signaling pathways play an important role in regulating AS160 in skeletal muscle in response to exercise. However, after moderate intensity exercise in lean older individuals (28), increases in Akt and AMPK phosphorylation correlated with changes in AS160 phosphorylation. In contrast, a recent study by Treebak et al. (32) suggested that, during exercise in humans, AMPK rather than Akt is more important for AS160 phosphorylation. In the present study, phosphorylation of both Akt Ser\(^{473}\) and AMPK Thr\(^{172}\) was increased immediately after exercise but then returned back to resting levels 3 h after exercise despite sustained AS160 phosphorylation. Further research will be required to determine the relative importance of these signaling pathways for AS160 regulation. However, as previously discussed for 14-3-3, it is possible that sustained activation of AS160 may occur in response to a brief transient interaction with Akt and/or AMPK. Alternatively, there may be timing differences associated with potential regulatory processes that act to dephosphorylate or inactivate AS160 and/or associated signaling pathways.

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phorylation and to understand the mechanisms by which distinct and/or coordinated phosphorylation of AS160 regulates GLUT4 translocation and glucose uptake in response to exercise and insulin.

It is generally well established that prior exercise increases insulin sensitivity and that this effect can persist for at least 48 h postexercise (19, 24, 36, 37). However, there are also a number of studies that have shown that insulin action is not always enhanced after an acute bout of exercise (5, 9, 14, 15). In the present study, no statistically significant increase (as determined by one-way ANOVA) was detected for whole body glucose uptake or glucose uptake when corrected for changes in insulin levels (insulin sensitivity index) immediately or 3 h after exercise. However, with a t-test, there was a trend \( P = 0.055 \) for an increase in the insulin sensitivity index as measured by the clamp performed 3 h after exercise compared with results shown for the resting trial. The lack of a statistically significant effect is likely due to a relatively small increase in glucose uptake, specifically in the exercising leg(s), which measures whole body glucose uptake. It also cannot be completely ruled out that the lack of a significant increase in insulin-stimulated glucose uptake after exercise was due to a relatively modest exercise intensity \(( \sim 60\% V\dot{O}_{2\text{peak}} )\).

In conclusion, the effect of acute aerobic exercise on AS160 phosphorylation in human skeletal muscle remains for at least 3 h after the completion of exercise. The 14-3-3 binding capacity of AS160 is also increased immediately after exercise, but this effect is not maintained 3 h after exercise completion. AS160 phosphorylation and the binding capacity of 14-3-3 were increased by insulin under resting conditions, but the AS160 phosphorylation and the binding capacity of 14-3-3 in response to changes in insulin levels (insulin sensitivity index) remained for at least 3 h after exercise. With a t-test, there was a trend \( P = 0.055 \) for an increase in the insulin sensitivity index as measured by the clamp performed 3 h after exercise compared with results shown for the resting trial. The lack of a statistically significant effect is likely due to a relatively small increase in glucose uptake, specifically in the exercising leg(s), which measures whole body glucose uptake. It also cannot be completely ruled out that the lack of a significant increase in insulin-stimulated glucose uptake after exercise was due to a relatively modest exercise intensity \(( \sim 60\% V\dot{O}_{2\text{peak}} )\).

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