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 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, 9.1 ± 0.5, and 9.2 ± 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 (10). It remains to be determined whether aerobic exercise can also regulate the interaction between AS160 and 14-3-3 and whether prior aerobic exercise can influence the subsequent response to insulin.

The aim of this study was to determine the effect of exercise and subsequent insulin stimulation on the regulation of AS160 and its binding capacity with 14-3-3 in human skeletal muscle. To examine this, hyperinsulinemic-euglycemic clamps were...
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 (V\textsubscript{O\textsubscript{2peak}}) as previously described (9). V\textsubscript{O\textsubscript{2peak}} 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, ~80% 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% V\textsubscript{O\textsubscript{2peak}}. 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\textsuperscript{-1}·min\textsuperscript{-1}). The insulin sensitivity index (M-to-I ratio) was calculated by dividing M by the mean insulin concentration (I; in nU/ml) during the same period of the clamp. M/I represents the amount of glucose disposed per unit of plasma insulin [mg·kg\textsuperscript{-1}·min\textsuperscript{-1}·(nU/ml)\textsuperscript{-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 each clamp (Fig. 1). Muscle samples were immediately frozen in liquid nitrogen for later analysis.

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 Na\textsubscript{2}PO\textsubscript{4}, 100 mM NaF, 2 mM Na\textsubscript{3}VO\textsubscript{4}, 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 [Ser\textsubscript{773} (no. 9271) and Thr\textsuperscript{308} (no. 9275)], phospho-Ser/Thr Akt substrate antibody (no. 9611), AMP-activated protein kinase (AMPK)-α2 (Thr\textsuperscript{172} (no. 2531), and acetyl-CoA carboxylase (ACC)-β (Ser\textsuperscript{211} (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) were from Upstate (Lake Placid, NY). Total 14-3-3 (K-19, no. sc-629) and anti-phosphotyrosine antibody (pY99, no. sc-7020) were from Santa Cruz Biotechnology (Santa Cruz, CA). The total AS160 antibody for immunoprecipitation was raised in sheep against the peptide CHPTDKKAKAGNKP (Cys + mouse residue 1295–1307), and total AMPK-α2 antibodies were kindly donated by Professor Graham Hardie (University of Dundee). ExtraAvidin peroxidase conjugate was 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 lysates (40–60 μg) were heated in SDS sample buffer, separated by gel electrophoresis using precast SDS-polycrylamide 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 lysates (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 Na\textsubscript{3}VO\textsubscript{4}, 5 mM Na\textsubscript{2}PO\textsubscript{4}, 0.27 mM sucrose, 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 (20, 21) was used in a manner similar to a primary antibody, with anti-DIG horseradish peroxidase as secondary antibody. All reagents were kindly donated by Professor Carol MacKintosh (University of Dundee).

Calculation and statistical analysis. Standards were included in all immunoblotting, and variation was accounted for by normalizing to control samples. All data are expressed as means ± SE. Statistical analysis was undertaken using a one- or two-way ANOVA for repeated measures. When ANOVA revealed significant differences,
RESULTS

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% \(\dot{V}O_2\text{peak}, 1.0 ± 0.0 \text{ arbitrary units}, 48.9 ± 1.9 \text{ l/min}, \) 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 3.7 ± 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 Ser\(^{473}\) (Figs. 2A and 4) increased significantly but not Akt Thr\(^{308}\) (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 Ser\(^{473}\) (Figs. 2A and 4) and Thr\(^{308}\) (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
insulin, there was a significant increase in the 14-3-3 binding capacity of AS160, although this was not influenced by prior exercise. Total 14-3-3 protein levels were similar in response to exercise and/or insulin (data not shown; representative blot in Fig. 2B).

**AMPK and ACC phosphorylation.** A single bout of aerobic exercise significantly increased AMPK Thr^{172} and ACC-β Ser^{221} phosphorylation immediately after exercise, which returned back to basal levels 3 h after exercise (Figs. 2A and 5). Insulin had no effect on AMPK Thr^{172} and ACC-β Ser^{221} phosphorylation. Total AMPK-α_{2} and ACC protein levels were similar in response to exercise and/or insulin (mean data not shown; representative blot in Fig. 2B).

**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.

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 decrease 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 Thr^{642}, the phosphorylation state of other sites, such as Ser^{341} and Ser^{318} (which cannot be efficiently detected by the phospho-Akt substrate antibody), may influence this interaction.

**Fig. 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.

**Fig. 4.** Insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation (top) and Akt Ser^{473} 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.
AMPK pathway also appears to be involved because 5-aminoimidazole-2-carboxamide-1-D-ribofuranoside, a pharmacological activator of AMPK, can increase AS160 phosphorylation in rodents (32). In response to acute 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 AMPK phosphorylation correlated with changes in the insulin-signaling pathway compared 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-D-ribofuranoside strongly phosphorylated AS160 at Ser\(^{588}\) and modestly at Thr\(^{642}\) and Ser\(^{751}\) in HEK-293 cells (8). As such, it is highly likely that a distinct pattern of phosphorylation on individual or multiple AS160 residues occurs in response to insulin and exercise. In future research studies, it will be important to utilize site-specific antibodies to determine the effects of insulin and exercise on AS160 phos-
phorylation and to understand the mechanisms by which dis-
tinct and/or coordinated phosphorylation of AS160 regulates
GLUT4 translocation and glucose uptake in response to exer-
cise 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 statisti-
cally significant effect is likely due to a relatively small increase
in glucose uptake, specifically in the exercising leg(s), which
could be difficult to detect with the hyperinsulinemic-eugly-
cemic clamp technique, 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 (~60% VO2peak).

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
response to insulin stimulation does not appear to be further
enhanced by prior exercise. Further research examining the
precise mechanism(s) by which insulin and exercise regulate
AS160 phosphorylation and 14-3-3 binding capacity may pro-
vide important information to explain the beneficial role of
exercise in the maintenance of health and in the prevention
and treatment of insulin resistance and associated disorders.

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