Aerobic Exercise Plus Weight Loss Improves Insulin Sensitivity and Increases Skeletal Muscle Glycogen Synthase Activity in Older Men

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The purpose of this study was to determine the effects of 6-month aerobic exercise training + weight loss (AEX + WL) on basal and insulin activation of glycogen synthase, basal citrate synthase activity, and Akt and AS160 phosphorylation in older, overweight/obese insulin-resistant men ($n = 14$; 63 ± 2 years; body mass index, $32 ± 3$ kg/m$^2$). Muscle samples of the vastus lateralis were collected before and during a 3-hour 80 mU/m$^2$/min hyperinsulinemic-euglycemic clamp. AEX + WL increased VO$_2$max by 11% ($p < .05$) and decreased body weight (−9%, $p < .001$). AEX + WL increased basal citrate synthase activity by 46% ($p < .01$) and insulin activation of independent (2.9-fold) and fractional (2.3-fold) activities (both $p < .001$) of glycogen synthase. AEX + WL had no effect on phosphorylation of Akt or AS160. Glucose utilization (M) improved 25% ($p < .01$), and the change tended to be related to the increase in insulin activation of glycogen synthase fractional activity ($r = .50, p = .08$) following AEX + WL. In summary, AEX + WL has a robust effect on insulin activation of skeletal muscle glycogen synthase activity that likely contributes to improved glucose utilization in older insulin-resistant men.

Key Words: Skeletal muscle glycogen synthase—Exercise—Insulin sensitivity—Weight loss.

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Insulin resistance and obesity are major yet modifiable risk factors for cardiovascular disease and type 2 diabetes. Obesity trends over the last decade continue to increase in men but not in women with current estimates of obesity indicating that almost 37% of men aged 60 and older are obese (1). In addition to obesity, physical inactivity contributes to the rise in diabetes rates with aging. Epidemiologic data indicate that new-onset type 2 diabetes in men can be reduced substantially by healthy lifestyle habits, namely weight loss and physical activity (2).

Exercise and weight loss are particularly important to implement in older, obese, insulin-resistant men to alter risk factors for diabetes. Aerobic exercise training alone or with weight loss (AEX + WL) improves insulin sensitivity (3–7). Potential mechanisms for improvements in insulin sensitivity may include an increase in the ability of insulin to activate glycogen synthase (GS), the activity of which is lower in insulin-resistant individuals (4,8). We recently reported that AEX + WL increased glucose utilization and insulin activation of GS in postmenopausal women with impaired glucose tolerance, whereas weight loss alone did not significantly change insulin activation of GS (4). Insulin stimulation of skeletal muscle Akt and Akt substrate of 160 kD (AS160) is also reduced in insulin-resistant states (9–11), and an increase in insulin’s effect could facilitate an increase in insulin sensitivity after AEX + WL. There are no studies examining the effects of a combined exercise and weight loss intervention on GS, Akt, and AS160 in older men. Our aim was to test the hypothesis that AEX + WL would increase muscle oxidative capacity (citrate synthase), Akt and AS160 phosphorylation, and insulin activation of GS, Akt, and AS160 to improve insulin sensitivity in obese, sedentary insulin-resistant older men.

Older men are less insulin sensitive by the glucose clamp than older women (12). A secondary aim of this study was to further understand a potential mechanism for the insulin resistance observed in men by comparing insulin activation of GS in older men with that in postmenopausal women (4). We hypothesized that reduced insulin activation of GS contributes to the greater insulin resistance in men compared with women.

Methods

All participants were healthy, overweight or obese (body mass index > 25 kg/m$^2$; range of 27–40 kg/m$^2$) men between
the ages of 54 and 77 years. Participants were sedentary (<20 minutes of exercise, 2 times/wk for the previous 6 months), had not smoked for more than 5 years, and were weight stable (<2.0 kg weight change in the past year). Participants were screened by medical history, physical examination, a 12-lead resting electrocardiogram, a graded exercise test, and a fasting blood profile to exclude liver, renal, or other hematological disease. The men were excluded from participation if they had evidence of poorly controlled hypertension (blood pressure > 160/90 mmHg), treated diabetes, abnormal cardiovascular response to a graded exercise test, orthopedic limitations that would affect physical activity, or other metabolic disorders. Participants were not on any medications that affect lipid or glucose metabolism. This study includes 14 men who had originally enrolled in a study of genetics and insulin sensitivity. Samples from women are a subset of a recent investigation from our lab (4). The Institutional Review Board of the University of Maryland approved all methods and procedures. Each participant provided written informed consent to participate in the study.

Study Design

Participants enrolled in a dietary-induced weight loss plus moderate- to high-intensity aerobic exercise intervention for 6 months. Participants received instruction in maintaining a weight-stable, Therapeutic Lifestyle Changes diet (13), by a registered dietitian 1 d/wk for 6–8 weeks, prior to baseline testing. Participants were weight stable on the Therapeutic Lifestyle Changes diet prior to baseline testing and were instructed to maintain this dietary composition throughout the study. The dietitian monitored compliance by weekly review of 7-day food records (or 24-hour recalls) using the American Diabetes Association exchange list system. During the 6-month weight loss intervention, the volunteers attended weekly weight loss classes led by a registered dietitian for instruction in the principles of hypocaloric diet according to the Therapeutic Lifestyle Changes guidelines. The volunteers were instructed to restrict their energy intake by 500 kcal/d.

Participants also exercised on a treadmill three times per week at our facility under the supervision of an exercise physiologist. Each exercise session included a 5- to 10-minute stretching and warm-up phase and a 5- to 10-minute cooling down phase. For the first ~4 weeks, participants exercised on the treadmill at ~50 VO2max for 20–30 continuous minutes as tolerated. Initially, time was progressed to 45–50 minutes followed by a progression of intensity to greater than 60% VO2max and up to 70% VO2max. This progression of intensity typically took approximately another 4 weeks based on each subject’s exercise tolerance. Exercise intensity, prescribed as a target heart rate range, was monitored using chest-strap heart rate monitors (Polar Electro Inc., Lake Success, NY). At the end of the 6-month program, participants were asked to continue their exercise during the final testing period. All metabolic testing at the end of the intervention was performed 36–48 hours after the last exercise session.

Body Composition

Height (cm) and weight (kg) were measured to calculate body mass index. Waist circumference was measured at the narrowest point superior to the hip. Fat mass, lean tissue mass, and bone mineral content were determined by dual-energy X-ray absorptiometry (Prodigy, LUNAR Radiation Corp., Madison, WI). A single computed tomography (Siemens Somatom Sensation 64 Scanner, Fairfield, CT) scan at L3–L5 region was used to determine visceral adipose tissue area and subcutaneous adipose tissue area and then analyzed using Medical Image Processing, Analysis and Visualization, v.7.0.0 (NIH Image Analysis Program). A second scan at the mid-thigh was used to quantify muscle area, total fat area, and low-density lean tissue area in Hounsfield units (3); values of the right leg were used in the statistical analyses. Six men did not undergo abdominal scans and two of these men also did not have a mid-thigh CT scan due to scheduling problems.

Maximal Oxygen Uptake

Maximal oxygen uptake (VO2max) was measured before and after the intervention by a progressive treadmill test to voluntary exhaustion as previously described (3). A valid VO2max was obtained when at least two of the following three criteria were met: (a) maximal heart rate greater than 90% of age predicted maximal heart rate (220 beats/min – age), (b) respiratory exchange ratio of greater than 1.10, and (c) plateau of VO2 (<200 mL/min change) with increasing work rate.

Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) and the glucose clamp were performed at least 7 days apart during pre- and posttesting. The OGTT was performed 36–48 hours after the last training bout. Blood samples were drawn before and at 30-min intervals for 2 hours after ingestion of 75 g of glucose and separated by centrifugation. Plasma glucose concentrations were measured using the glucose oxidase method (2300 STAT Plus, YSI, Yellow Springs, OH). Plasma insulin was measured by RIA (Millipore Inc., St. Charles, MO). Glucose and insulin total area under the curve (glucose and insulin) were calculated by the trapezoidal method. Homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) was calculated ([fasting insulin [μU/mL] × fasting glucose [mmol/L]])/22.5 as well as HOMA for insulin secretion (HOMA-β) as (20 × fasting insulin [μU/mL])/[fasting glucose [mmol/L] − 3.5] as described by Matthews (14).
Participants were classified as having normal glucose tolerance or impaired glucose tolerance by the American Diabetes Association criteria (15).

**Hyperinsulinemic-Euglycemic Clamp**

All participants were weight stable (±2%) for at least 2 weeks prior to the glucose clamp before and after the interventions. Participants were provided all meals as a eucaloric diet for 2 days before the clamp by a registered dietitian to control nutrient intake (3). All testing was performed in the morning after a 12-hour overnight fast and 36–48 hours after the last training bout. Glucose utilization (M) was determined using the hyperinsulinemic-euglycemic clamp procedure (16,17). An intravenous catheter was inserted into an antecubital vein for infusion of insulin and glucose. Arterialized blood was obtained from a dorsal heated hand vein (18). After a priming dose of insulin, humulin insulin (Eli Lilly, Indianapolis, IN) was infused at a constant rate of 80 mU/min for 3 hours. Plasma glucose levels were measured at 5-minute intervals using the glucose oxidase method (Beckman Instruments) and maintained at basal levels with a variable infusion of 20% glucose, which was adjusted according to a computerized algorithm. Samples were also obtained at 10-minute intervals during the clamp for measurement of plasma insulin levels by RIA (Millipore Inc., St. Charles, MO). Insulin sensitivity was expressed as M/I, which represents the amount of glucose metabolized per unit of plasma insulin (I), and was calculated by dividing the mean glucose utilized by the mean insulin concentration during the last hour of the clamp for each subject.

**Skeletal Muscle Biopsies**

All 14 men underwent a vastus lateralis biopsy under local anesthesia, and samples were utilized for assays described subsequently dependent on having a sufficient amount of tissue obtained. We conducted the GS assay on skeletal muscle tissue from 12 men and the Western blot analyses on a different set of 10 men. Skeletal muscle samples were immediately freeze clamped in tongs frozen in liquid nitrogen and stored at −80°C. Muscle samples were lyophilized for 48 hours and then dissected free of obvious connective tissue, fat, and blood.

**GS assay (n = 12).**—Independent, total, and fractional activities of GS were determined as previously described (4). The independent activity of GS (nonphosphorylated) was determined in the presence of a physiological concentration of glucose-6-phosphate (0.1 mmol/L), an allosteric activator of GS. The total activity of GS was determined in the presence of a saturating glucose-6-phosphate concentration (10 mmol/L). The total activity of GS is the sum of the independent and dependent forms of GS. GS fractional activity is the ratio of independent activity to total activity and is expressed as a percent. The fractional (relative) and independent (actual) activities of GS provide a measure of the amount of GS in the active form.

**CS assay (n = 12).**—One milligram of lyophilized micro-dissected skeletal muscle was homogenized in 150 µL ice-cold buffer containing (in mmol/L) 250 sucrose, 10 Tris–HCl, 1 ethylenediaminetetraacetic acid, pH 7.4, and protease inhibitors (Roche 11836170001). CS activity was measured by continuous spectrophotometric rate determination as described (19) using 10 µL of the 1:150 homogenate in a final volume of 1 mL. All skeletal muscle enzyme activities were corrected for total protein content (Coomassie Plus, Pierce).

**Phosphorylated Akt (pSer473) and AS160 (pThr642; n = 10 due to limited amount of tissue).**—Protein levels in skeletal muscle were determined by Western blot. Samples were homogenized in ice-cold buffer containing (in mmol/L) 100 HEPES, 100 NaCl, 10 Na_2PO_4, 50 NaF, 10 ethylenediaminetetraacetic acid, 10 MgCl_2, 10 Na_2VO_4, 10% glycerol, 1% Triton, protease inhibitor, and phosphatase inhibitors I and II (Sigma-Aldrich, St. Louis, MO). Insoluble materials were removed by centrifugation at 12,000g for 10 minutes at 4°C. An aliquot containing 40 µg of protein was solubilized in Laemmli sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and electrophotoreically transferred to a PVDF membrane. Phospho-Akt (pSer^{473}) and Phospho-AS160 (pThr^{642}) were detected with rabbit polyclonal antibodies (Cell Signaling Technology, Danvers, MA, and BioSource International, Camarillo, CA, respectively), followed by horseradish peroxidase–labeled goat anti-rabbit IgG (Cell Signaling Technology, Danvers, MA). Proteins were visualized with SuperSignal West Femto Chemiluminescent Substrate (Thermo Fisher Scientific, Rockford, IL) and quantified using a Fluorchem imager (Cell Biosciences, Santa Clara, CA). All samples from the same subject were analyzed on the same blot/membrane. Individual samples were adjusted for total protein concentrations. Phosphoproteins were expressed relative to specific protein concentrations. For presentation, quantifications of protein phosphorylation are shown relative to the preintervention basal fasting sample in arbitrary units.

**Statistics**

Paired Student’s t-tests were used to test the effect of the intervention for all variables within men. For the comparison between men and women, Student’s t-tests for independent samples were used. Pearson correlations were used to assess relationships between key variables. Data were analyzed using SPSS version 18 (IBM, Armonk, NY). All data are presented as mean ± standard error of the mean. Statistical significance was set at p < .05.
RESULTS

Aerobic Capacity, Body Composition, and Glucose Metabolism

At baseline, participants were sedentary (VO\textsubscript{2max}; 20.1 ± 1.0 mL/kg/min), obese (body mass index: 31.7 ± 1.0 kg/m\textsuperscript{2}), and insulin resistant (HOMA-IR: 3.85 ± 0.40). HOMA-IR was negatively associated with M (r = -0.67, p < .01) and M/I (r = -0.74, p < .05) at baseline. The AEX + WL intervention increased VO\textsubscript{2max} (expressed in L/min) by 11% (p < .05), decreased body weight by 9% (p < .001), fat mass by 18% (p < .001), with a significant reduction in %body fat (p < .001) and small but significant decrease in FFM (p < .05). AEX + WL also resulted in a 17% reduction in waist circumference (p < .01), 29% decrease in visceral fat area (156.2 ± 15.1 vs. 109.2 ± 14.0 cm\textsuperscript{2}, p < .05), and 23% decrease in subcutaneous fat area (278.5 ± 36.7 vs. 228.0 ± 46.3 cm\textsuperscript{2}, p < .01). In addition, mid-thigh low-density lean tissue area decreased by 14% (41.3 ± 3.1 vs. 35.2 ± 3.2 cm\textsuperscript{2}, p < .05) and mid-thigh subcutaneous fat area decreased by 25% (143.0 ± 22.9 vs. 101.3 ± 14.0 cm\textsuperscript{2}, p < .05) with no significant change in muscle area (180.0 ± 19.6 vs. 162.8 ± 19.0 cm\textsuperscript{2}) (Table 1).

At baseline, nine men had normal glucose tolerance, four had impaired glucose tolerance, and one had untreated type 2 diabetes by G\textsubscript{120} during the OGTT. Of the five men with abnormal responses to the OGTT, two of the four with impaired glucose tolerance reverted to normal glucose tolerance and the one with type 2 diabetes reverted to impaired glucose tolerance. In the entire group, fasting glucose decreased by 4% (p < .05), but changes in fasting insulin, G\textsubscript{120}, insulin \textsubscript{AIC}, and glucose \textsubscript{AUC} did not reach statistical significance. There was a 42% increase in HOMA-\beta (2.74 ± 0.44 vs. 3.62 ± 0.50, p < .01), but the 16% decrease in HOMA-IR was not statistically significant (3.85 ± 0.40 vs. 3.13 ± 0.45, p = .10). Glucose utilization (M) increased by 25% (p < .01) and M/I increased by 35% (p < .01).

Effects of AEX + WL on Skeletal Muscle Enzyme Activities and Protein Levels

Citrate synthase activity increased 46% after the intervention (0.083 ± 0.007 to 0.118 ± 0.009 μmol/min·mg protein; p < .01). Fractional and independent activities of GS increased with insulin before (p < .01) and after (p < .001) AEX + WL (Table 2). The effect of insulin to increase fractional and independent activities of GS was 2.3- and 2.9-fold, respectively, after AEX + WL (p < .001, Figure 1). GS total activity was not affected by insulin before or after AEX + WL. Basal activities of GS (fractional, independent, and total) were not affected by AEX + WL (Table 2).

There were no changes in basal Akt and AS160 phosphorylation after AEX + WL (Figure 2a and b). Hyperinsulinemia during the clamp increased phosphorylation of Akt before and after the intervention (6- and 7-fold, respectively, p < .01). There was also an increase in the phosphorylation of AS160 with insulin before and after AEX + WL (1.9-fold; p < .05 and 1.5-fold; p = .09). However, the insulin-stimulated increase in Akt and AS160 phosphorylation (Figure 2a and b) was similar before and after AEX + WL. Therefore, there was no difference in insulin-stimulated phosphorylation of Akt or AS160 following AEX + WL.

Relationships Between Changes in Metabolic and Muscle Variables and Insulin Sensitivity After AEX + WL

The increase in M with AEX + WL was related to the reduction in visceral fat area (r = -0.87, p < .01). There were no significant relationships between changes in M or M/I and the changes in subcutaneous abdominal fat, intramuscular fat, or VO\textsubscript{2max}. The decrease in HOMA-IR was related to an increase in VO\textsubscript{2max} (r = -.55, p = .05) as was the increase in HOMA-\beta (r = .55, p = .05). The increase in CS (percent change) also correlated with the increase in VO\textsubscript{2max} (r = .63, p < .05). The change in GS fractional activity (insulin-stimulated minus basal) was related to M before and after AEX + WL (r = .70 and r = .58, respectively, p < .05), and the change (post-pre) in insulin-stimulated GS fractional activity tended to correlate with the change in M (r = .50, p = .08). The change in GS fractional activity was not related to either the change in weight, visceral fat, subcutaneous abdominal fat, or VO\textsubscript{2max}. The changes in Akt or AS160 protein levels with AEX + WL did not correlate with changes in M or VO\textsubscript{2max}.

Comparison of M and GS Between Older Men and Women

We had a unique opportunity to further understand the insulin resistance of these older men by comparing their...
data to that of women who underwent identical glucose clamp procedures, GS measurements (assays were run simultaneously for both groups), and AEX + WL interventions as described in a recently published study (4). The postmenopausal women were of similar age (men vs. women, 63 ± 2 vs. 60 ± 1 years) and VO$_{2\text{max}}$ (19.5 ± 0.9 vs. 20.4 ± 0.8 mL/kg/min). As in the current study of men, the comparison group included both normal glucose tolerance and impaired glucose tolerance women ($n = 31$).

At baseline, the men had a 56% higher basal GS fractional activity and 40% higher basal GS total activity than the women (fractional activity, 14 ± 2 vs. 9 ± 1%, $p < .05$; total activity, 14 ± 1 vs. 10 ± 1, $p < .05$). Also at baseline, the men had 62% lower rates of glucose utilization (Figure 3a, $p < .0001$) and a reduced ability to increase GS fractional activity with insulin compared with the women (Figure 3b, $p < .05$). Glucose utilization was significantly related to the ability of insulin to increase GS fractional activity in both men and women ($r = .36$, $p < .05$, Figure 4). Following AEX + WL, the men had a greater percent change in the ability of insulin to increase GS fractional activity compared with the women ($p < .05$).

**DISCUSSION**

Overweight and obese older men are at risk for the development of insulin resistance, glucose intolerance, and type 2 diabetes. Our study indicates that a combined aerobic exercise and weight loss lifestyle intervention improves insulin sensitivity in these men with concomitant increases in insulin-stimulated GS fractional activity. Further, the reduced ability to increase GS fractional activity with insulin prior to AEX + WL likely explains the greater insulin resistance of older men compared with similar-aged women.

There are only a few studies examining the effects of aerobic exercise alone on insulin-stimulated GS activity. Our prior work in older obese men showed that a 6-month aerobic exercise program was more effective at improving insulin activation of GS than a 6-month resistive training program (12). Other investigators showed that basal and insulin-stimulated GS fractional activity did not change after a short aerobic exercise intervention (8 weeks) in younger obese men and women (20). Likewise, in young healthy men ($n = 8$), a 3-week one-legged training program did not increase insulin’s effect on GS fractional activity (21). Our data indicate that the 6-month AEX + WL intervention in middle-aged and older, insulin-resistant men resulted in
Figure 2. (a) Basal (open bar) and insulin-stimulated (closed bar) Akt phosphorylation at baseline/pre and after 6 months AEX + WL (n = 10) *p < .01 basal versus insulin stimulated. Representative blots are shown above graph. (b) Basal (open bar) and insulin-stimulated (closed bar) AS160 phosphorylation at baseline/pre and after 6 months AEX + WL (post; n = 10) *p < .05 basal versus insulin-stimulated. Representative blots are shown above graph.
a 2.9-fold increase in insulin-stimulated GS independent activity and a 2.3-fold increase in GS fractional activity, without a change in GS total activity. Because our exercise duration was three to eight times longer and the men were more insulin resistant at baseline, it is conceivable that both the duration of training and initial level of insulin sensitivity are important components to consider in whether exercise training induces changes in insulin activation of GS.

It is unlikely that the weight loss component of the intervention had any effect on insulin activation of GS. We did not observe a significant change in insulin activation of GS after a 6-month weight loss intervention (−8% body weight) in postmenopausal women (4). This is consistent with other reports wherein an 11% weight loss by a very low-calorie diet did not have an effect on insulin-stimulated GS activity in obese nondiabetic participants (22). Others also fail to show significant changes in GS activity after substantial weight loss (23). In addition, the change in insulin activation of GS activity was not related to the change in body weight in our study. Therefore, we believe that the increases in independent and fractional activities (percent change above baseline) of GS are primarily due to the exercise component of the intervention.

The 2.5- to 3-fold increase in GS fractional activity at baseline in postmenopausal women (4) was higher than the 2-fold increase observed in these older men despite identical clamp protocols and similar steady-state insulin levels. The women also had higher rates of glucose utilization, higher basal citrate synthase activity (unpublished data), and lower mid-thigh low-density lean tissue area compared with the men despite similar age and fitness level. Reduced mitochondrial function (oxidative capacity) could contribute to the greater ectopic fat accumulation and insulin resistance in the older men versus older women, similar to what Petersen and coworker (24) postulated for older versus younger individuals. Because we observed that the increase in GS fractional activity with insulin was associated with M in our previous study of women (4) and in the current study of men, we believe that this reduced insulin activation of GS fractional activity contributes to the insulin resistance in these men.

Akt regulates the activation of AS160 that mediates insulin-stimulated GLUT4 translocation (25). Insulin stimulates phosphorylation of Akt and AS160 in young participants (21). In this study, we confirm this with 6- to 7-fold increases in Akt and almost 2-fold increases in AS160 in older insulin-resistant men. These actions are reduced in type 2 diabetes, impaired glucose tolerance, and insulin-resistant states (26–28). As far as the effects of exercise and diet, most of the published work has utilized the rat model and not human skeletal muscle. One recent study (29) found that a 2-week diet with weight loss of ~4 kg improved insulin sensitivity in a small group of obese women and men but did not change basal phospho-AS160 (pSer711) or phospho-Akt (pSer473 and Thr308). Although insulin-stimulated
biopsies were not obtained, this study would suggest that weight loss alone does not alter Akt activation. In one other study in young healthy men, one-legged exercise training increased insulin-stimulated activity of Akt1 and phosphorylation of AS160 (21). Although we found changes in insulin activation of GS, the lack of significant changes in the ability of insulin to phosphorylate Akt and AS160 after AEX + WL may be concordant with the findings of Frosig and coworker (21) who suggested that Akt1 is not a major kinase regulating the response of GS activity to insulin stimulation in human skeletal muscle.

There are several strengths to this study including sophisticated measure of insulin sensitivity, carefully characterized participants at baseline and follow-up, robust increase in aerobic capacity by VO2max and CS, and large weight loss in a group of very sedentary insulin-resistant men, typical of age-related obesity-associated insulin resistance. Limitations of this study are the small sample size, including both normal and glucose-intolerant men, which did not enable us to compare the effect of the intervention by glucose tolerance status, and the lack of a control group of AEX or WL alone. However, the significance of our findings, despite these limitations, strengthens the importance of GS as a key mediator of the metabolic improvement in glucose metabolism and insulin sensitivity in obese, sedentary middle-aged and older men.

The results of our study indicate that the improvement in insulin sensitivity (M or HOMA) after AEX + WL in older men may be attributed, in part, to the reduction in visceral fat and increased fitness. Our major new finding is that significant changes in insulin activation of GS were related to insulin-mediated glucose uptake after AEX + WL independent of Akt and AS160 in these older, obese men. Future studies should address the role of protein phosphatases in regulating insulin activation of GS after AEX + WL in older individuals.

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**References**


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