Impairments in Site-Specific AS160 Phosphorylation and Effects of Exercise Training

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The purpose of this study was to determine if site-specific phosphorylation at the level of Akt substrate of 160 kDa (AS160) is altered in skeletal muscle from sedentary humans across a wide range of the adult life span (18–84 years of age) and if endurance- and/or strength-oriented exercise training could rescue decrements in insulin action and skeletal muscle AS160 phosphorylation. A euglycemic-hyperinsulinemic clamp and skeletal muscle biopsies were performed in 73 individuals encompassing a wide age range (18–84 years of age), and insulin-stimulated AS160 phosphorylation was determined. Decrement in whole-body insulin action were associated with impairments in insulin-induced phosphorylation of skeletal muscle AS160 on sites Ser-588, Thr-642, Ser-666, and phospho-Akt substrate, but not Ser-318 or Ser-751. Twelve weeks of endurance- or strength-oriented exercise training increased whole-body insulin action and reversed impairments in AS160 phosphorylation evident in insulin-resistant aged individuals. These findings suggest that a dampening of insulin-induced phosphorylation of AS160 on specific sites in skeletal muscle contributes to the insulin resistance evident in a sedentary aging population and that exercise training is an effective intervention for treating these impairments. Diabetes 62:3437–3447, 2013

Skeletal muscle plays a prominent role in whole-body glucose regulation and is considered the primary target for insulin-mediated glucose uptake (1). In skeletal muscle, the binding of insulin to the insulin receptor initiates a signaling process that results in the translocation of the insulin-sensitive glucose transporter (GLUT4) to cell surface membranes and the facilitated diffusion of glucose into the cell (2). The complex nature of this process is evident by data indicating normal activation of proximal signaling components, including Akt, despite overt insulin-resistant conditions imposed by lipid infusion (3), fasting (4), obesity (5), and diabetes (5). Such findings suggest that elements downstream of Akt may be more closely related to insulin action. In skeletal muscle, the Akt substrate of 160 kDa (AS160), also known as TBC1D4, a Rab guanosine triphosphatase-activating protein (GAP), is currently recognized as the most distal signaling step associated with insulin-mediated glucose transport. In the basal state, the GAP guanosine triphosphatase-activating domain of AS160 is hypothesized to maintain Rab proteins in their inactive form, allowing AS160 to colocalize and retain GLUT4 in intracellular vesicles (6). In response to insulin, AS160 becomes phosphorylated on a number of Akt consensus sequences, suppressing its GAP activity and resulting in the translocation of GLUT4 to the plasma membrane (6–9). The functional importance of phosphorylated AS160 is evident because a mutation in one or more phosphorylation sites results in a reduction in insulin-stimulated GLUT4 translocation (9–11).

Insulin-stimulated AS160 phosphorylation may be impaired in insulin-resistant conditions because the insulin-induced phosphorylation of AS160 is diminished with type 2 diabetes (12,13), polycystic ovary syndrome (14), and fasting (4). Site-specific impairments in AS160 phosphorylation (Ser-318, Ser-588, and Ser-751) have recently been reported (13) with type 2 diabetes, suggesting that certain phospho-specific sites may have greater implications in insulin resistance. However, it is not evident whether the site-specific regulation of AS160 is evident and consistent across insulin-resistant conditions in human skeletal muscle.

The inhibitory mechanisms regulating AS160 phosphorylation remain obscure. In adipocytes, the transcriptional coregulator, receptor interacting protein 140 (RIP140), has been reported to interact with AS160, impeding the ability of Akt to phosphorylate AS160 (15). It remains unknown whether RIP140 impairs AS160 phosphorylation through a similar mechanism in the skeletal muscle of insulin-resistant individuals.

Endurance- (16–18) and strength-oriented (19,20) exercise training can both improve insulin sensitivity and are recommended as a means of intervention/prevention for insulin resistance. However, data examining the effect of exercise training on AS160 phosphorylation in human skeletal muscle is sparse. Some findings indicate that short-term endurance training (3 weeks or less) was not sufficient to increase insulin-stimulated AS160 phosphorylation in young, healthy (21), obese, nondiabetic (22), or diabetic individuals (22). Unfortunately, conclusions from these studies (21,22) are limited based on their use of the anti-phospho-Akt substrate (PAS) antibody, which is thought to only recognize phosphorylation of AS160 on site Thr-642 (9,23).

The insulin resistance typically evident in middle- to older-aged individuals is multifaceted and involves increases in...
overall and central adiposity and a reduction in cardiopulmonary fitness as well as the effect(s) of chronological age itself (1,24–31). The main objectives of the current study were to 1) determine if the insulin resistance evident in sedentary, middle- to older-aged individuals is associated with impaired site-specific phosphorylation of AS160 in human skeletal muscle and 2) to determine whether strength- and/or endurance-oriented exercise training could rescue these impairments.

RESEARCH DESIGN AND METHODS

Experimental design. Study 1 used a cross-sectional design encompassing younger to older individuals to determine if insulin action is associated with a decrement in insulin signaling at the level of AS160. Study 2 determined if a specific exercise training modality (endurance or strength training) could effectively ameliorate the insulin resistance evident in sedentary, insulin-resistant aged individuals by enhancing the most distal component of insulin signal transduction. Figure 1 provides an overview of the experimental design.

Study 1—Cross-sectional study examining insulin action and distal insulin signaling in human skeletal muscle

Participants. Specifically recruited for this study were 73 participants (41 women, 32 men) comprising a wide age range (18–84 years). Physical characteristics of the subjects are provided in Table 1. All participants were nonsmokers and participated in less than 1 h/week of organized physical activity, as assessed by a standardized questionnaire. In an attempt to study a representative population, inclusion required that a participant’s BMI be between the 25th and 75th percentile for his or her decade of age (32). Excluded were individuals with heart disease, diabetes, endocrine, and/or metabolic disorders, and those taking lipid-altering medication. Premenopausal women were excluded from the young, endurance group due to noncompliance (Fig. 1).

Preliminary testing. Cardiorespiratory fitness was measured with an incremental, maximal treadmill test (33), with expired gases analyzed continuously (TrueMax 2400; ParvoMedics, Sandy, UT) to determine VO2peak. Body composition was measured by dual X-ray absorptiometry, and circumference measurements of the waist, hip, and thigh were obtained with a spring-loaded measuring tape.

Euglycemic-hyperinsulinemic clamp and muscle biopsies. Subjects reported to the laboratory at 0700 after a 12-h overnight fast. A 2-h euglycemic-hyperinsulinemic clamp was used to determine insulin action and elicitation of insulin signaling, as previously described (18,34). Briefly, a primed insulin (Humulin; Eli Lilly, Indianapolis, IN) infusion was performed for 10 min (starting at 313 mU · m−2 · min−1), followed by a continuous infusion of insulin at a sub-maximal dosage of 100 mU · m−2 · min−1. Blood samples were obtained every 5 min, centrifuged, and analyzed for serum glucose (YSI 2300 STAT Plus Glucose and Lactate Analyzer; YSI Inc., Yellow Springs, OH), and the glucose infusion rate was adjusted as needed to maintain euglycemia. Blood plasma was obtained every 10 min and stored at −80°C for the subsequent analysis of plasma insulin (Access Immunoassay System; Beckman Coulter, Fullerton, CA). A steady-state M-value was determined from the final 20 min of the clamp (35).

A biopsy specimen was obtained from the vastus lateralis with the percutaneous muscle biopsy technique at baseline and at 60 min of the clamp. The 60-min time point was selected because we have previously reported that components of insulin signal transduction (PE3-kinase activation and Akt Ser-473 phosphorylation) appeared to be maximally activated at this time (36–38). Tissue samples were immediately frozen in liquid nitrogen for subsequent analyses.

Western blot and immunoprecipitation procedures. Skeletal muscle was homogenized and protein content determined as previously described (39,40). For Western blot analyses, muscle lysate (30–100 μg cellular protein) was separated by SDS-PAGE, electrophoresed on polyvinyldiene difluoride membranes (Millipore, Billerica, MA), and probed overnight with Cell Signaling (Beverly, MA) antibodies for PAS, phospho (p)Akt-Ser473 (recognizes Akt-Ser472/473/474), AS160, Akt2, and cyclooxygenase IV (COXIV). Membranes were also probed for pAS160-Thr642 (Millipore), pAS160-Ser666 (Millipore), pAS160-Ser588 (Symansis NZ Ltd, Timaru, New Zealand), GLUT4 (Affinity BioReagents, Golden, CO), RIP140 (Santa Cruz Biotechnology, Santa Cruz, CA), AS160 (Abcam, Cambridge, MA), and phospho-specific antibodies for AS160 at sites Ser-318 and Ser-751, as previously described (23,41). Proteins were visualized by horseradish peroxidase-conjugated 1:10000 antibodies and ECL SuperSignal (Pierce Biotechnology, Rockford, IL) exposed to X-ray film. All samples were normalized to a control sample on each gel, and phosphorylation levels were additionally normalized to total protein after membranes were stripped, as previously reported (41), and reprobed with the corresponding antibody for total protein. For immunoprecipitation, lysates (200 μg) were incubated at 4°C overnight with Cell Signaling Technology antibodies for AS160 or Akt2 and for 3 h with protein A Sepharose beads (GE Healthcare Biosciences Corp., Piscataway, NJ). Supernatant portions from samples were removed and immunocomplexes analyzed with Western blotting.

Study 2—Effect of exercise training on distal insulin signaling Participants. Of the 73 subjects recruited for the cross-sectional study, 45 volunteered for the experiment examining the effects of exercise training on insulin signal transduction. Inclusion criteria required participants to be ≤35 years of age (young) or ≥55 years of age (aged). These individuals were then randomized into a 12-week endurance- (n = 12 young, n = 11 aged) or strength-training (n = 11 young, n = 11 aged) program. Two individuals were subsequently excluded from the young, endurance group due to noncompliance (Fig. 1).

FIG. 1. Overview of experimental design. Young, ≤35 years (range 18–35); aged, ≥55 years (range 55–84).
Changes in characteristics of young and aged individuals before and after endurance training

![Image of a page from a document with text about participant characteristics and changes in insulin action and distal insulin signaling.](image-url)

**TABLE 1**

Participant characteristics for cross-sectional study

| Variable                  | Young (n = 10) | Aged (n = 11) |
|---------------------------|---------------|---------------|
| Age (range)               | 24.4 ± 1.6 (18–34) | 69.0 ± 2.2† (57–84) |
| Sex (n)                   |               |               |
| Female                    | 5             | 5             |
| Male                      | 5             | 6             |
| Insulin action            |               |               |
| M-value (mg/kg/min)       | 7.9 ± 0.8     | 9.8 ± 0.7*    |
| Body composition          |               |               |
| Mass (kg)                 | 69.8 ± 2.2    | 69.2 ± 2.5    |
| BMI (kg/m²)               | 23.8 ± 0.9    | 23.5 ± 0.7    |
| Body fat (%)              | 25.5 ± 3.7    | 23.6 ± 3.5*   |
| Lean body mass (kg)       | 47.4 ± 2.8    | 48.8 ± 3.1*   |
| Thigh circumference (cm)  | 48.7 ± 1.6    | 48.3 ± 1.5    |
| Waist-to-hip ratio        | 0.78 ± 0.02   | 0.77 ± 0.02   |
| Performance               |               |               |
| Peak isokinetic leg extension (N) | 569 ± 72 | 449 ± 47    |
| Peak isokinetic leg flexion (N) | 396 ± 49 | 252 ± 32†   |
| 1-RM leg press (kg)       | 119 ± 9       | 121 ± 10      |
| 1-RM chest press (kg)     | 49 ± 6        | 54 ± 8*       |
| Fasting blood chemistry   |               |               |
| Glucose (mg/dL)           | 84.3 ± 2.4    | 78.6 ± 2.8*   |
| Insulin (IU/mL)           | 4.6 ± 1.0     | 3.7 ± 1.1*    |

Data are presented mean ± SEM (range). †P < 0.05 vs. pretraining. *P < 0.05 vs. aged.
TABLE 3
Changes in characteristics of young and aged individuals before and after strength training

| Variable                  | Young (n = 11) | Aged (n = 11) |
|--------------------------|---------------|--------------|
|                          | Pretraining   | Post-training| Pretraining   | Post-training |
| Age (years)              | 23.6 ± 1.5 (20–35) | 69.3 ± 2.7† (55–82) | 6.1 ± 0.7† | 6.9 ± 0.7†‡ |
| Sex (n)                  |               |              |               |              |
| Female                   | 4             | 6            |               |              |
| Male                     | 7             | 5            |               |              |
| Insulin action           |              |              |               |              |
| M-value (mg/kg/min)      | 9.4 ± 0.8     | 10.8 ± 1.0*  | 6.1 ± 0.7† | 6.9 ± 0.7†‡ |
| Body composition         |              |              |               |              |
| Mass (kg)                | 71.8 ± 3.5    | 73.3 ± 3.3*  | 76.4 ± 3.7 | 76.7 ± 3.7* |
| BMI (kg/m²)              | 23.5 ± 0.7    | 25.3 ± 1.2   | 26.8 ± 1.1 | 26.9 ± 1.1  |
| Body fat (%)             | 22.9 ± 3.7    | 22.1 ± 3.7   | 36.9 ± 2.6† | 36.2 ± 2.7† |
| Lean body mass (kg)      | 53.0 ± 3.6    | 53.9 ± 3.4*  | 45.2 ± 3.3 | 46.2 ± 3.3* |
| Thigh circumference (cm) | 50.9 ± 1.1    | 51.4 ± 1.0   | 49.0 ± 1.1 | 48.5 ± 1.5  |
| Waist-to-hip ratio       | 0.76 ± 0.02   | 0.75 ± 0.02  | 0.87 ± 0.03†| 0.87 ± 0.03†|
| Performance              |              |              |               |              |
| V0∙peak (ml/kg/min)      | 38.2 ± 2.1    | 41.5 ± 2.8*  | 20.6 ± 1.8† | 21.7 ± 1.9‡ |
| Peak isokinetic leg extension (N) | 718 ± 70  | 874 ± 72* | 500 ± 43† | 534 ± 36† |
| Peak isokinetic leg flexion (N) | 413 ± 38 | 530 ± 41* | 292 ± 24† | 331 ± 29* |
| 1-RM leg press (kg)      | 121 ± 11      | 148 ± 17*    | 81 ± 12†  | 103 ± 91*  |
| 1-RM chest press (kg)    | 54 ± 10       | 58 ± 9*      | 31 ± 5    | 37 ± 5*    |
| Fasting blood chemistry  |              |              |               |              |
| Glucose (mg/dL)          | 84.1 ± 2.5    | 80.1 ± 2.8*  | 92.3 ± 2.8 | 89.5 ± 2.3* |
| Insulin (IU/mL)          | 4.8 ± 1.1     | 3.7 ± 0.9*   | 5.1 ± 1.1  | 3.7 ± 1.0* |

Data are presented mean ± SEM (range) or as indicated. Statistics performed on absolute values. †P < 0.05 age main effect. ‡P < 0.05 training main effect.
General adaptations to exercise training. Differences in whole-body insulin action (M-value), body composition, \( \text{VO}_2\text{peak} \), and fasting blood chemistries were evident between the young and aged groups before initiating the 12 weeks of exercise training (Tables 2 and 3). With endurance training, there was a reduction in lean body mass, which resulted in no change in overall body mass (Table 2). Endurance training also increased insulin action (M-value), \( \text{VO}_2\text{peak} \), and 1-repetition maximum (RM) chest press and decreased fasting plasma glucose and insulin (Table 2). Strength training increased 1-RM leg press, 1-RM chest press, and peak isokinetic leg flexion strength in young and insulin-resistant aged participants and also increased lean and total body mass and \( \text{VO}_2\text{peak} \) (Table 3). Insulin action also increased irrespective of group, whereas fasting plasma glucose and insulin concentrations decreased in response to strength training (Table 3). Skeletal muscle COXIV protein, a marker of mitochondrial content, increased in response to endurance training (~30%) but not with strength training (Supplementary Fig. 3). Analysis of 3-day diet records revealed no significant changes in caloric...
intake (total, protein, carbohydrate, and fat) during the training period.

**AS160 phosphorylation.** In agreement with the correlation analyses (Supplementary Fig. 1), reductions in insulin-stimulated Ser-588, Thr-642, Ser-666 (P < 0.05), and PAS (P = 0.06) phosphorylation were evident in insulin-resistant aged individuals in the pretraining, sedentary state (Fig. 4). Endurance training increased insulin-stimulated AS160 phosphorylation of PAS by ~60% in young and ~75% in insulin-resistant aged individuals (P < 0.05, Fig. 5A), whereas AS160 Ser-588 phosphorylation increased ~25% in both groups (P < 0.05, Fig. 5B). There was a significant interaction (P < 0.05) for AS160 Thr-642 in response to endurance training as the aged individuals increased AS160 Thr-642 phosphorylation by ~57% (P < 0.05, Fig. 5C), whereas no significant changes were observed in the young individuals. There was a tendency (P = 0.07) for insulin-stimulated Ser-666 phosphorylation to increase with endurance training in the aged subjects (Fig. 5D). No changes with endurance training were evident in insulin-stimulated Ser-751 and Ser-318 (data not shown). Strength training increased insulin-stimulated AS160 phosphorylation of PAS by ~75% (P < 0.01, Fig. 6A) in both groups, whereas insulin-stimulated Thr-642 phosphorylation increased by ~33% and ~73% in young and aged individuals (P < 0.05, Fig. 6C), respectively. In addition, strength training increased insulin-stimulated Ser-666 phosphorylation of AS160 by ~100% (Fig. 6D) in the insulin-resistant aged group. There were no changes in Ser-588 (Fig. 6B), Ser-751 (data not shown), or Ser-318 (data not shown) with strength training.

Exercise training had no effect on AS160 protein content (data not shown). Basal phosphorylation levels of PAS were significantly reduced (~50%, P < 0.01) in response to strength training, which contributed to the significant training effect (Fig. 6A). Exercise training had no other effect on basal AS160 phosphorylation levels. Endurance training increased GLUT4 (~10% for young and ~15% for aged, P < 0.05; Supplementary Fig. 4), whereas strength training had no effect. Multiple regression analysis indicated that exercise-induced changes in phosphorylation of PAS, Ser-588, Thr-642, and Ser-666 accounted for 28% of the variance in the improvement in insulin action (P < 0.05).

Before exercise training, Akt2 protein levels did not differ between the groups (data not shown). Endurance training increased Akt2 protein levels by ~60% in the young group (P < 0.01), with a similar trend in the aged individuals (P = 0.08). In response to strength training, Akt2 protein levels demonstrated a trend for an increase (P = 0.08). Neither age nor exercise training had an effect on Akt2 Ser-473 phosphorylation when normalized to protein content (Supplementary Fig. 5).

**DISCUSSION**

In the current study, we show for the first time that insulin-stimulated AS160 phosphorylation, measured by the PAS antibody, and specific phosphorylation at sites Ser-588, Thr-642, and Ser-666 are impaired in human skeletal muscle in conjunction with the decrement in insulin action typical with advancing age and a sedentary lifestyle (Figs. 2 and 4). Impaired insulin-mediated AS160 phosphorylation has been reported in other insulin-resistant conditions, including type 2 diabetes (12) and polycystic ovary syndrome (14), using the PAS antibody. The PAS antibody
may recognize multiple phosphorylation sites on AS160; however, current research suggests this antibody is limited to only recognizing AS160 phosphorylation on Thr-642 (9,23). More recently, site-specific impairments were identified in patients with type 2 diabetes (Ser-318, Ser-588, and Ser-751) (13) and in healthy individuals after fasting-induced insulin resistance (Ser-588 and Ser-751) (4). The current data (Figs. 2 and 4), in combination with other data (4,13), provide the important information that an impairment in Ser-588 phosphorylation appears to be consistent in human skeletal muscle across conditions inducing insulin resistance. In contrast, other AS160 sites demonstrate differential phosphorylation patterns, possibly as a product of kinases and phosphatases being regulated by the severity or the pathology of insulin resistance. Collectively, these findings show that conditions of whole-body insulin resistance are linked with site-specific impairments in AS160 and provide novel insight into a signaling impairment located distally in the insulin-signaling cascade.

In an attempt to investigate cellular mechanisms that could contribute to the impaired AS160 phosphorylation, we examined RIP140 expression and its association with AS160. In adipocytes, the binding of RIP140 to AS160 results in reduced glucose uptake, likely as a result of RIP140 impeding the ability of Akt to inactivate GAP activity on AS160 (15). The current finding that insulin-resistant aged individuals had a higher amount of RIP140 complexed with AS160 (Fig. 3 B) provides novel evidence that the impairment in AS160 phosphorylation may be linked to the association of AS160 with RIP140, which in turn induces insulin resistance (Fig. 3C).

In an attempt to gain an understanding of factors that may regulate site-specific phosphorylation on AS160, we performed regression analyses using variables linked with insulin action. Body fat percentage was the best predictor of Ser-588 phosphorylation, whereas basal plasma insulin levels proved to be the best predictors of Thr-642 phosphorylation, suggesting these phosphorylation sites may be differentially regulated. In agreement with these findings, in vitro experiments in adipocytes demonstrated that insulin-stimulated phosphorylation of Thr-642 occurs much more rapidly than Ser-588, and hierarchical clustering analysis revealed that Thr-642 did not cluster with Ser-588 (42). Taken together, this information provides insight into potential regulatory mechanisms of AS160 phosphorylation; however, we acknowledge that regression analyses only imply relationships and that additional variables not measured in the current study may also play a role in the regulation of site-specific AS160 phosphorylation.

Of the two Akt isoforms expressed in skeletal muscle (Akt1 and Akt2), Akt2 is considered crucial for glucose uptake in skeletal muscle (43). In relation to upstream signaling of AS160, we recognize that phosphorylation of both Akt Ser-473 and Thr-308 is required for the full activation of Akt; however, the current study was limited to Akt2 Ser-473 based on results in human skeletal muscle indicating that insulin-stimulated Akt2-Ser473 phosphorylation (as opposed to Akt-Thr308) was closely related to AS160-PAS phosphorylation and glucose uptake (43). Insulin-stimulated Akt2-Ser473 phosphorylation was not associated with insulin-resistant aged individuals in the current study; however, Sharma et al. (44), recently reported reduced insulin-stimulated Akt2-Thr308 phosphorylation in the soleus of aged (25-month) compared with adult (9-month) rats; therefore, we cannot conclusively state that all Akt sites were preserved with insulin resistance.

Exercise training has long been recognized as a method to improve insulin action (16–19) (Tables 2 and 3). The effect of exercise training on insulin-stimulated AS160 phosphorylation has been sparsely addressed, particularly...
in regards to phospho-specific sites. Previous research reported that insulin-mediated AS160 phosphorylation increased in healthy young men after 3 weeks of one-legged endurance-oriented exercise training; however, these effects were negated when phosphorylation was normalized to AS160 protein content (21). In addition, O’Gorman et al. (22) reported that short-term endurance training (7 days) was not sufficient to increase insulin-stimulated AS160 phosphorylation in obese, nondiabetic, or diabetic individuals. However, conclusions from these studies (21,22) are limited based on the use of the PAS antibody.

A key finding in the current study was that decrements in specific insulin-stimulated AS160 phosphorylation sites were improved with exercise training (Figs. 5 and 6), with the exception of AS160 Ser-588 (Fig. 6B), which did not appear to be responsive to strength training (Fig. 6B). Vind et al. (13) previously reported increased insulin-induced AS160 phosphorylation on Ser-588 in type 2 diabetic patients, but not in nondiabetic control subjects, in response to 10 weeks of endurance training, suggesting that this site may be particularly responsive to endurance exercise in insulin-resistant populations. From our
cross-sectional data, we determined that body fat percentage was the best predictor of AS160 Ser-588 phosphorylation. Body fat percentage was reduced in response to endurance, but not strength training, which could explain why improvements in Ser-588 phosphorylation were only evident with this training modality. Protein kinase Cζ (PKCζ) activity has been hypothesized to regulate AS160 Ser-588 phosphorylation (42), and endurance training has been reported to increase skeletal muscle PKCζ activity (45). Although speculative, it is plausible to suggest that our endurance-training program improved PKCζ activity, which could in part explain improvements in insulin-stimulated AS160 Ser-588 phosphorylation.

Both modes increased PAS, Thr-642, and Ser-666 (P = 0.07 with endurance training) phosphorylation in the aged group, indicating the effectiveness of exercise in treating insulin resistance. The clinical relevance of our findings is that either endurance or strength training appears to improve insulin action through similar mechanisms in relation to insulin signaling at the level of AS160. This finding may provide relevant information in terms of therapeutic treatments for insulin-resistant conditions.

FIG. 6. Phosphorylation levels of skeletal muscle AS160 PAS (n = 11, young; n = 11, aged) (A), Ser-588 (n = 11, young; n = 11, aged) (B), Thr-642 (n = 10, young; n = 10, aged) (C), and Ser-666 (n = 10, young; n = 10, aged) (D) in response to insulin before and after 12 weeks of strength training in young (23.6 ± 1.5 years of age, range 20–35 years, white bars) and aged (69.3 ± 2.7 years, range 55–82 years, black bars) individuals. Data are presented as fold change in phosphorylation levels normalized to total AS160 protein levels. The line above the bars represents the main effect for age (short bar) or training (long bar). Data are mean ± SEM. E: Representative blots using AS160 phospho-specific antibodies and total protein in young and aged individuals under noninsulin (B) and insulin-stimulated (I) conditions. For PAS, AS160 was initially immunoprecipitated and then blotted with the PAS antibody. *P < 0.05 vs. pretraining. †P < 0.05 vs. young at that comparable time.
Consistent with other studies examining human subjects over a wide life span (1,24,26,30,46), our data demonstrated that whole-body insulin action declined with age (Supplementary Fig. 1). The nature of this age-related insulin resistance has been well-studied and likely involves a number of contributing factors, including increased abdominal adiposity (30), declining cardiorespiratory fitness (29,31), and chronological age itself (29), all of which were evident in our population (Table 1). Despite reports suggesting that the effect of chronological age is negated when adjusting for BMI (26), adiposity (27), or physical inactivity (28), our data indicate that chronological age was an independent predictor of whole-body insulin action, which is in agreement with the findings of at least one other study (29).

A limitation of the current study was that muscle fiber typing was not performed. Animal studies have reported greater age-related impairments in glucose uptake in slow-twitch compared with fast-twitch muscle (44,47), despite the apparent preservation of type I fiber cross-sectional area with aging (48). Likewise, 12 weeks of endurance or strength training has been associated with increases in type I (49) and type II (50) fiber area, respectively, independent of age (48,49). Therefore, the age- or exercise-related differences in AS160 phosphorylation in our current study could possibly have been influenced by changes in muscle fiber type. In addition, despite previous research suggesting maximal AS160 phosphorylation occurs at 60 min of an euglycemic-hyperinsulinemic clamp (36,38), we cannot exclude the possibility that the rate of site-specific AS160 phosphorylation was influenced by either age and/or exercise training.

In conclusion, the findings of the current study indicate for the first time that deficits in whole-body insulin action evident with the aging process and a sedentary lifestyle are associated with reduced insulin-stimulated phosphorylation of specific AS160 sites (Thr-642, Ser-588, Ser-666, and PAS). With respect to intervention/prevention, 12 weeks of endurance- or strength-oriented exercise training increased whole-body insulin action and rescued impairments in AS160 phosphorylation. Collectively, these findings suggest that decrements in the ability of insulin to phosphorylate specific sites on skeletal muscle AS160 contribute to insulin resistance and that exercise training is an effective treatment option to counteract these impairments.

ACKNOWLEDGMENTS
This work was supported by National Institutes of Health grants AG-025205 and DK-56112 to J.A.H. and by the Danish Medical Research Council and The Novo Nordisk Research Foundation to J.F.P.W. J.T.T. was supported by a postdoctoral fellowship from The Danish Agency for Science, Technology and Innovation, Denmark. The Novo Nordisk Foundation Center for Basic Metabolic Research is an independent Research Center, based at the University of Copenhagen, Denmark, and partially funded by an unconditional donation from the Novo Nordisk Foundation (www.metabol.ku.dk). No other potential conflicts of interest relevant to this article were reported.

L.A.C. and J.A.H. conceived and developed the experiments. L.A.C. and J.V.M. collected and analyzed the exercise training data. L.A.C., C.A.N., D.N.C., M.S.D., C.J.T., and J.A.H. collected and analyzed euglycemic-hyperinsulinemic data. C.A.N., D.N.C., M.S.D., and J.A.H. obtained skeletal muscle biopsies. L.A.C. generated Western blot data. L.A.C., J.F.P.W., J.T.T., and J.A.H. analyzed Western blot data. L.A.C. and J.A.H. wrote the manuscript. All authors provided comments and approved the final version. L.A.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Rita Bowden, RN, and Angela Clark, RN, East Carolina University, for their clinical expertise during the euglycemic-hyperinsulinemic clamp procedure; and Dr. Kristen Boyle, Dr. Benjamin Bikman, Todd Weber, MS, and Gina Battaglia, MS, East Carolina University, for assisting with data collection during the euglycemic-hyperinsulinemic clamp procedure. The authors are grateful to the graduate students in the Kinesiology Department at East Carolina University for monitoring the exercise training programs of the participants and express their appreciation toward the study participants.

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