Increased Skeletal Muscle Capillarization Independently Enhances Insulin Sensitivity in Older Adults After Exercise Training and Detraining

Intramuscular signaling and glucose transport mechanisms contribute to improvements in insulin sensitivity after aerobic exercise training. This study tested the hypothesis that increases in skeletal muscle capillary density (CD) also contribute to exercise-induced improvements in whole-body insulin sensitivity (insulin-stimulated glucose uptake per unit plasma insulin [M/I]) independent of other mechanisms. The study design included a 6-month aerobic exercise training period followed by a 2-week detraining period to eliminate short-term effects of exercise on intramuscular signaling and glucose transport. Before and after exercise training and detraining, 12 previously sedentary older (65 ± 3 years) men and women underwent research tests, including hyperinsulinemic-euglycemic clamps and vastus lateralis biopsies. Exercise training increased $V_{O2\max}$ (2.2 ± 0.2 vs. 2.5 ± 0.2 L/min), CD (313 ± 13 vs. 349 ± 18 capillaries/mm$^2$), and M/I (0.041 ± 0.005 vs. 0.051 ± 0.007 μmol/kg fat-free mass/min) ($P < 0.05$ for all). Exercise training also increased the insulin activation of glycogen synthase by 60%, GLUT4 expression by 16%, and 5' AMPK-α1 expression by 21%, but these reverted to baseline levels after detraining. Conversely, CD and M/I remained 15% and 18% higher after detraining, respectively ($P < 0.05$), and the changes in M/I (detraining minus baseline) correlated directly with changes in CD in regression analysis (partial $r = 0.70$; $P = 0.02$). These results suggest that an increase in CD is one mechanism contributing to sustained improvements in glucose metabolism after aerobic exercise training.

Aging is associated with physical inactivity and lifestyle behaviors that contribute to vascular dysfunction and microvascular rarefaction. Skeletal muscle capillary density (CD) is lower in older compared with younger adults (1,2) and is associated with glucose intolerance and lower insulin-stimulated glucose uptake (3–5). This reduction in CD decreases the available surface area for diffusion of glucose into the interstitium (6,7) and may also limit insulin action (8). Therefore, strategies to increase skeletal muscle CD may ameliorate age-related declines in insulin sensitivity and glucose tolerance by enhancing muscle perfusion to promote glucose uptake and metabolism.

Interventions including aerobic exercise (AEX) training and weight loss reduce insulin and glucose responses to glucose tolerance tests (9,10), increase insulin-stimulated glucose uptake (11–13), and reduce progression to type 2 diabetes among people with insulin resistance (14,15). AEX training also increases skeletal muscle CD (16,17); we recently showed that AEX training and weight loss–induced increases in skeletal muscle CD are directly associated with improvements in glucose tolerance and insulin-stimulated glucose uptake in older adults with impaired glucose tolerance (18). It is, however, often difficult to distinguish the contribution of CD to improvements in insulin sensitivity...
independent of intramuscular signaling mechanisms and significant weight loss.

Increases in skeletal muscle GLUT4 expression (11,19–23), AMPK expression (24,25), and insulin activation of glycogen synthase (GS) (13,26–28) all contribute to increase insulin sensitivity after AEX training. While AEX training enhances all of these mechanisms, the effects are not all long-lasting. For example, 5–10 days of detraining causes a reversal of exercise-induced increases in skeletal muscle GS activity (29) and GLUT4 expression (19–22). Conversely, exercise training–induced increases in skeletal muscle CD are largely maintained for up to 12 weeks of detraining in endurance-trained subjects (30,31). Thus, we anticipated that 2 weeks of detraining after 6 months of AEX training would eliminate AEX-induced improvements in intramuscular signaling and glucose transport protein expression, allowing us to test the hypothesis that longer-lasting increases in CD independently contribute to improvements in insulin sensitivity in older adults. We assessed the responses of skeletal muscle CD and insulin sensitivity to AEX training and detraining, and we measured GS activity, citrate synthase (CS) activity, GLUT4 and AMPKα1 expression, and pAktSer473 as benchmark indices of the intramyocellular responses to training and detraining in older adults.

RESEARCH DESIGN AND METHODS

Subjects
Men and postmenopausal women 50–80 years of age were recruited from the Baltimore, Maryland, area to participate in studies examining metabolic responses to AEX training. All subjects were previously sedentary (self-reported exercise <20 min on ≈2 days/week), nonsmokers, and reported no previous diagnosis of diabetes or cardiovascular disease. Subjects were excluded if they had liver or renal disease, chronic pulmonary disease, cancer, or a physical impairment that would limit exercise participation. The women in the study had not menstruated for at least 1 year and were not prescribed hormone replacement therapy. Subjects taking medications for hypertension or dyslipidemia were included if they were medically stable and if medications were known not to affect glucose metabolism. This study was approved by the institutional review board at the University of Maryland School of Medicine, and all subjects provided written informed consent.

Study Design
Before and after 6 months of AEX training, subjects underwent research testing consisting of body composition measurement, oral glucose tolerance tests (OGTTs), maximal exercise tests, and hyperinsulinemic-euglycemic clamps with basal and insulin-stimulated vastus lateralis biopsies (Fig. 1). After AEX training, subjects underwent each metabolic test 24–36 h after one of their usual exercise sessions. Subjects then stopped all exercise training for 2 weeks and repeated research testing at the detraining time point. Subjects were counseled to maintain body weight during all phases of the study.

For the AEX training and detraining interventions, subjects exercised on motorized treadmills at the Baltimore Veterans Affairs Medical Center Geriatric Research Education and Clinical Center exercise facility 3 times/week, 45 min/session, for 6 months. Exercise intensity was prescribed as a target heart rate range using the Karvonen formula (32), and heart rate was monitored during exercise using heart rate monitors (Polar Electro Inc., Lake Success, NY). AEX training began at a volume of three 20-min sessions/week at 50% of heart rate reserve and gradually increased to 45 min at ∼75% of heart rate reserve, a level maintained for >4 months.

Research Testing

Hyperinsulinemic-Euglycemic Clamp
Insulin-stimulated glucose uptake per unit plasma insulin (M/I) was measured as an index of insulin sensitivity after a 12-h overnight fast using a 3-h hyperinsulinemic-euglycemic glucose clamp (33,34) with an insulin infusion rate of 555 pmol/m²/min. Data are reported as M/I during the third hour of the clamp (micromoles of glucose infused per kilogram of fat-free mass per picomole of plasma insulin per minute). Plasma glucose concentrations were analyzed at 5-min intervals using the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were determined by radioimmunoassay (Millipore, St. Charles, MO). The mean insulin and glucose concentrations during the clamp were 1,255 ± 49 pmol/L and 5.1 ± 0.1 mmol/L, respectively, and did not differ across time points (P ≥ 0.4). Subjects were provided with all meals for 2 days preceding the clamp to control nutrient intake.

Vastus Lateralis Biopsies
Bergstrom needles (Stille, Solna, Sweden) were used to obtain biopsies from the right vastus lateralis, approximately 12–14 cm above the patella, as previously described (35). Muscle samples were obtained immediately before and 2 h after beginning insulin infusion. One portion of each muscle sample was rapidly embedded in optimal cutting temperature–tragacanth gum mixture and frozen for histochemical analyses. The remaining muscle was immediately freeze-clamped using tongs frozen in liquid nitrogen and stored at −80°C; this portion of the sample was lyophilized for 48 h and then dissected free of connective tissue, fat, and vascular cells before protein and enzyme assays.
Skeletal Muscle CD
Muscle was sectioned to a thickness of 14 μm, and capillaries were identified by immunohistochemistry using Ulex europaeus agglutinin I to detect endothelial cells and mouse anticolonlagen IV antibody to detect muscle fiber perimeters, as previously described (18). Stained muscle sections were imaged (Eclipse Ti; Nikon Instruments Inc., Melville, NY) and analyzed using NIS Elements software (Nikon Instruments Inc., Melville, NY). CD (capillaries per square millimeter of muscle cross-sectional area) was quantified using more than 50 fibers for each sample (mean 70 ± 2 fibers/sample); sampling a larger number of fibers does not improve the estimation of capillarization in human muscle (36).

GLUT4, AMPKα1, pAkt, and Akt Expression
Skeletal muscle was homogenized in lysis buffer (20 mmol/L HEPES, 100 mmol/L NaCl, 1.5 mmol/L MgCl2, 0.1% Triton X-100, and 20% glycerol) containing 1 mmol/L dithiothreitol and 1 tablet of complete mini EDTA-free Protease Inhibitor Cocktail (Roche, Nutley, NJ). After homogenization, samples were kept on ice for 20 min, followed by centrifugation for 10 min at 20,000g. The supernatant was collected and stored at −80°C. GLUT4 and AMPKα1 expression were measured in basal samples; Akt and pAktSer473 were measured in both basal and insulin-stimulated samples to assess insulin action. Total protein was determined using Pierce BCA Protein Assay Kits (Thermo Scientific, Waltham, MA), and either 20 μg (Akt and pAktSer473) or 30 μg (GLUT4 and AMPKα1) of total protein was loaded on SDS polyacrylamide gels. After gel electrophoresis, proteins were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA) and blocked with 5% nonfat dry milk for 1 h at room temperature. Membranes were washed briefly with Tris-buffered saline and incubated with primary antibodies detecting GLUT4 (anti-GLUT4, 5 μg/mL; R&D Systems, Minneapolis, MN), AMPKα1, total Akt, or pAktSer473 (anti-AMPKα1, anti-Akt, and anti-pAktSer473, 1:1,000; Cell Signaling Technology, Danvers, MA). β-Actin (anti-β-actin, 1:1,000; Cell Signaling Technology) was used for total protein normalization. After primary antibody incubation, membranes were washed with Tris-buffered saline and incubated with horseradish peroxidase–linked anti-rabbit IgG or antimouse IgG (1:1,000; Cell Signaling Technology) secondary antibodies for 1 h at room temperature. For GLUT4 and AMPKα1, target bands were visualized using SuperSignal West Dura Chemiluminescent Substrate (Thermo Scientific) and detected using the GeneGnome imaging system (Syngene, Frederick, MD). Band densities were quantified using densitometry (ImageJ software; National Institutes of Health, Rockville, MD). For pAkt, the signal was detected by adding an enhanced chemiluminescence horseradish peroxidase substrate (SuperSignal West Dura Extended Duration Substrate; Thermo Scientific), and for total Akt, the signal was detected by adding Clarity enhanced chemiluminescence (Bio-Rad, Hercules, CA). Protein bands were visualized using the Bio-Rad Image Laboratory System. All protein expression data are expressed as arbitrary units.

GS Activation, Glycogen Content, and CS Activity
GS independent, total, and fractional activities were determined in muscle samples as previously described (28). The independent and total activities of GS (nonphosphorylated) were determined in the presence of physiological (0.1 mmol/L) and saturating (10 mmol/L) concentrations of glucose-6-phosphate, respectively. GS fractional activity is the ratio of independent activity to total activity, expressed as a percentage. The difference in GS fractional activity between the insulin-stimulated and basal conditions is a measure of insulin activation of GS. Glycogen content was measured in the same supernatant as GS. Supernatant was boiled for 5 min, then centrifuged at 13,000g for 5 min. This supernatant (10 μL) was used for fluorometric determination of glycogen using the Glycogen Assay Kit (Sigma-Aldrich, St. Louis, MO). For CS activity, 1 mg of muscle was homogenized in 150 μL of ice-cold buffer containing 250 mmol/L sucrose, 10 mmol/L Tris-HCl, 1 mmol/L EDTA (pH 7.4), and complete mini EDTA-free Protease Inhibitor Cocktail (Roche). CS activity was measured by continuous spectrophotometric rate determination, as previously described (37). All measures were corrected for total protein content using Coomassie Plus (Thermo Scientific) and assayed in triplicate.

Maximal Oxygen Consumption
VO2max was measured by indirect calorimetry during a graded treadmill exercise test to maximal effort on a motorized treadmill. VO2max was defined as the highest oxygen consumption value obtained for a 30-s increment. Attainment of VO2max was verified by standard physiological criteria (respiratory exchange ratio >1.10 or a plateau in VO2 with an increase in workload).

Body Composition
Height and weight were measured to calculate BMI. Fat mass and fat-free mass were measured by DXA (Prodigy; Lunar Radiation Corp., Madison, WI). Areas of intra-abdominal fat (IAF) and subcutaneous abdominal fat (SAF) were determined by computed tomography of the L4-L5 region (Somatom Sensation 64; Siemens, Fairfield, CT) and Medical Image Processing, Analysis and Visualization software version 7.0.0 (National Institutes of Health, Bethesda, MD). Computed tomography data were not available for one subject because of a technical problem.

OGTTs and Lipid Profiles
Subjects underwent 2-h OGTTs after a 12-h overnight fast. A catheter was placed in an antecubital vein and blood samples were drawn for glucose, insulin, and lipoprotein-lipids. After ingestion of a 75-g glucose solution, blood samples were drawn every 30 min for 2 h for measurement.
of glucose and insulin concentrations. Blood samples were centrifuged and plasma triglycerides and lipoprotein-lipid concentrations were analyzed using an automated colorimetric assay, as previously described (38). Plasma samples were stored at −80°C for analysis of glucose concentrations (2300 STAT Plus; YSI, Yellow Springs, OH) and insulin by radioimmunoassay (Millipore).

Statistical Analyses
The primary study outcomes were M/I and skeletal muscle CD. Secondary outcomes included GLUT4, AMPKα1, Akt, and pAktSer473 protein expression, skeletal muscle glycogen content, GS fractional activity, CS activity, $V_{O_2\text{max}}$, fasting plasma glucose, plasma glucose response to an OGTT, and body composition. Data are presented as means ± SEM. Statistical analyses were performed using SPSS version 12.0 (IBM, Armonk, NY). Repeated-measures ANOVA was used to test for differences in outcome variables before and after exercise training and detraining. Regression analyses were used to test for associations between M/I and other variables. A type I error rate of $\alpha = 0.05$ was selected, and two-tailed probabilities are reported for all analyses.

RESULTS

Subject Characteristics
Subjects were all middle-aged to older men and women with no recent history of exercise. At baseline, the BMI of subjects ranged from normal weight to class I obesity (24–32 kg/m²). Participants all had normal or well-controlled blood pressure (112 ± 3 mmHg systolic; 70 ± 2 mmHg diastolic) and lipoprotein-lipid concentrations (Table 1). Classified by baseline OGTTs, six subjects had normal glucose tolerance (NGT) and six had impaired glucose tolerance (three men and three women) (Table 1).

Table 1—Subject characteristics and responses to AEX training and detraining

|                      | Baseline | 6-month exercise period | Detraining |
|----------------------|----------|-------------------------|------------|
| Sex (male/female), n | 7/5      | —                       | —          |
| Age (years)          | 65 ± 3   | 81 ± 5*                 | 81 ± 5*    |
| Weight (kg)          | 83 ± 5   | 27.0 ± 1.3*             | 27.1 ± 1.4*|
| Body fat (%)         | 35.3 ± 2.8| 33.3 ± 3.0*             | —          |
| Fat mass (kg)        | 29.9 ± 3.3| 28.0 ± 3.6*             | —          |
| Fat-free mass (kg)   | 53.3 ± 3.4| 52.9 ± 3.2              | —          |
| IAF area (cm²)       | 153.9 ± 15.5| 145.9 ± 14.9            | —          |
| SAF area (cm²)       | 291.6 ± 50.4| 294.2 ± 53.3            | —          |
| $V_{O_2\text{max}}$ (mL/kg/min) | 26.2 ± 1.7| 31.2 ± 2.3†              | 29.3 ± 1.9† |
| $V_{O_2\text{max}}$ (L/min)   | 2.24 ± 0.22| 2.57 ± 0.27†             | 2.44 ± 0.26*|
| Fasting insulin (pmol/L) | 83 ± 9   | 74 ± 7                  | 89 ± 8     |
| Fasting plasma glucose (mmol/L) | 5.15 ± 0.10| 5.26 ± 0.10              | 5.16 ± 0.08|
| $G_{120}$ (mmol/L)   |          |                        |            |
| All subjects         | 7.40 ± 0.53| 7.13 ± 0.61              | 6.50 ± 0.54*|
| NGT (n = 6)          | 5.92 ± 0.09| 6.20 ± 0.63              | 5.63 ± 0.57|
| IGT (n = 6)          | 8.89 ± 0.57| 7.69 ± 0.66              | 7.36 ± 0.80*|
| Total cholesterol (mmol/L) | 4.55 ± 0.28| 4.47 ± 0.31              | 4.44 ± 0.25|
| LDL cholesterol (mmol/L) | 2.78 ± 0.28| 2.74 ± 0.30              | 2.67 ± 0.26|
| HDL cholesterol (mmol/L) | 1.16 ± 0.09| 1.23 ± 0.09*             | 1.23 ± 0.12|
| Triglycerides (mmol/L) | 1.35 ± 0.14| 1.12 ± 0.13*             | 1.18 ± 0.13*|

Data are means ± SEM. *Significant difference from baseline, $P < 0.05$. †Significant difference from baseline, $P < 0.01$. $G_{120}$, 120-min postprandial glucose; IGT, impaired glucose tolerance.

Metabolic Effects of Exercise Training and Detraining
AEX training increased $V_{O_2\text{max}}$ (liters per minute) by 15% ($P = 0.006$), the majority of which (10%) was maintained after 2 weeks of detraining ($P = 0.03$; Table 1). A goal of the study was for subjects to maintain body weight, and although statistically significant, the subjects lost only 2 ± 1 kg of body weight after exercise training ($P = 0.04$) (Table 1). Subjects did lose a small amount of body fat; however, there were no changes in IAF or SAF after exercise training (Table 1). Six-month AEX training increased M/I by 25%, and an 18% increase was maintained after 2 weeks of detraining ($P = 0.03$, ANOVA) (Fig. 2). Among the entire group of subjects, a 12% decrease in 120-min postprandial glucose was detected after detraining, largely because of subjects with impaired glucose tolerance who reduced 120-min postprandial glucose by 13–17% ($P = 0.02$) (Table 1). Half of the subjects with impaired glucose tolerance reverted to NGT after the interventions. Subjects had 17% lower triglyceride concentrations and 6% higher...
although pAktSer473 increased in response to insulin at training and detraining (expression was higher than baseline after exercise and AMPK AEX training increased GLUT4 protein expression by 16% Metabolism Mechanisms Affecting Skeletal Muscle Glucose Effects of Exercise Training and Detraining on Exercise training (Fig. 3) approached significance, and GS activation reverted to baseline levels after detraining (67 ± 4% vs. 70 ± 5% vs. 69 ± 4% type I fibers, respectively; P = 0.80) and skeletal muscle fiber area did not either (4,732 ± 421 vs. 4,961 ± 460 vs. 4,784 ± 494 μm², respectively; P = 0.89), indicating that increases in CD were not a result of changes in muscle fiber size or type. Thus, of the measured factors that could contribute to increases in M/I, CD was the only variable to increase and remain significantly higher after exercise training and detraining. We assessed the correlation between these variables in a regression model accounting for sex and small changes in percentage body fat, finding that the change in M/I (detraining minus baseline) correlated directly with the change in CD (partial r = 0.70, P = 0.02; model R = 0.82, P = 0.02) (Fig. 5).

DISCUSSION
This study demonstrates that in previously sedentary older adults at risk for developing type 2 diabetes, an AEX training–induced increase in skeletal muscle CD is one mechanism contributing to improvements in insulin sensitivity. Our previous study (18) showed that changes in insulin sensitivity after AEX training with significant weight loss (8%) are related to improvements in CD; however, that study did not allow us to distinguish the independent effects of exercise training and weight loss or the effects of CD independent of other mechanisms. By minimizing the effects of weight loss and reversing acute exercise-induced changes in intramuscular mechanisms with detraining, the current study could better assess the ability of increased CD to improve M/I. Because skeletal muscle is responsible for the majority of insulin-stimulated glucose uptake, identifying microvascular mechanisms that mediate skeletal muscle uptake of glucose has significant implications for the prevention and treatment of insulin resistance and type 2 diabetes.

The design of this study allowed us to distinguish the chronic and acute effects of AEX training on metabolic outcomes. For example, 6 months of AEX training increased VO2max by 15% in previously sedentary older subjects, and VO2max decreased slightly after detraining, but two-thirds of the increase remained. While the change in CS activity (a marker of mitochondrial enzyme activity) was not statistically significant, the magnitude of the change is likely physiologically significant because it is similar to those
reported in previous studies using AEX training (16) and detraining (39), and the pattern of changes in CS activity was consistent with the changes in \( V_O^{max} \). This suggests that part of the reduction in \( V_O^{max} \) after detraining may be attributed to the reversal of short-term changes in mitochondrial enzyme activity. The sustained improvement in \( V_O^{max} \) after detraining is likely attributable to the effects of chronic exercise training on the cardiovascular system, including increases in CD to enhance oxygen delivery.

After 6 months of AEX training, M/I increased by 25%. This corresponded with an increase in skeletal muscle CD, increases in GLUT4 and AMPK \( \alpha_1 \) expression, and an increase in insulin activation of GS. The direction and magnitude of these changes are similar to previous studies of diabetes.diabetesjournals.org Prior and Associates

**Figure 3**—Changes in skeletal muscle GLUT4 and AMPK \( \alpha_1 \) (A), Akt (B), and pAkt\(^{\text{Ser}473}\) (C) expression, as well as the pAkt\(^{\text{Ser}473}\)-to-Akt ratio (D), after exercise training and detraining. Levels of GLUT4, AMPK \( \alpha_1 \), total Akt, and pAkt\(^{\text{Ser}473}\) proteins were determined in vastus lateralis by Western blot analyses. Representative Western blots are shown directly above the corresponding bars in each graph. Protein expression levels were determined by densitometry of the chemiluminescence signal and are expressed as arbitrary units (AU). Data are presented as means ± SEM (\( n = 12 \)). *Significant difference from baseline, \( P < 0.05 \). †Significant difference from basal condition (without insulin stimulation) at the same time point, \( P < 0.01 \). mo, month.

| Table 2—GS and CS activities |
|-----------------------------|
|                            | Baseline | 6-month exercise period | Detraining |
|                            | Basal | Insulin stimulated | Basal | Insulin stimulated | Basal | Insulin stimulated |
| GS activity Fractional (%) | 7.6 ± 1.0 | 21.9 ± 3.0† | 6.7 ± 1.0 | 29.1 ± 5.4† | 7.5 ± 1.4 | 20.3 ± 2.7† |
| Independent (nmol/min/mg protein) | 0.46 ± 0.14 | 1.52 ± 0.26† | 0.48 ± 0.07 | 1.94 ± 0.28† | 0.52 ± 0.1 | 1.67 ± 0.23† |
| Total (nmol/min/mg protein) | 6.4 ± 1.6 | 7.1 ± 0.9 | 8.5 ± 1.9 | 8.2 ± 1.3 | 7.3 ± 0.9 | 8.1 ± 1.0 |
| CS activity (μmol/min/mg protein) | 0.16 ± 0.03 | 0.21 ± 0.03 | 0.15 ± 0.03 |
| †Significant effect of insulin (difference from basal), \( P < 0.01 \). |
CD (16–18), GLUT4 (11,23), AMPKα1 (24,25), and insulin activation of GS (13,27,28). These findings indicate that a number of intracellular and extracellular mechanisms likely contribute to improvements in M/I immediately following AEX training; however, insulin action did not seem to contribute to this increase (Fig. 6). Although total Akt protein expression increased after exercise training, basal or insulin-stimulated pAktSer473 did not change after exercise training or detraining. This finding is consistent with previous studies of AEX training with or without weight loss among nondiabetic young and older subjects (27,40,41). Furthermore, Wasserman showed that in response to insulin infusion similar to that used in this study, resistance to muscle glucose uptake shifts away from insulin signaling and glucose transport and toward glucose delivery (rev. in 42), so it is not surprising that insulin action may not have contributed to the increase in M/I seen in this study. There is evidence that a larger transcapillary barrier is required to limit insulin delivery and action compared with that required to limit glucose delivery and uptake (42,43). In this study, the lower CD at baseline may not have been low enough to limit insulin delivery and action; therefore, increases in CD would not be expected to further enhance insulin action but may reduce the barrier to glucose delivery to increase M/I. One experimental model did show that occlusion of capillaries reduces insulin action (44); however, it is likely that the occlusion of capillaries created a significant barrier to insulin delivery in that study.

After detraining, M/I decreased ~7% but still remained 18% higher than baseline levels. The maintenance of higher skeletal muscle glycogen content after detraining shows that the increase in M/I was not an artifact of glycogen depletion that could have enhanced insulin-stimulated glucose uptake (45). The partial decrease in M/I with detraining paralleled the decrease in GS activation, GLUT4 expression, and AMPKα1 expression to baseline levels. These findings are consistent with previous reports showing that GS activation and GLUT4 expression decrease in humans within days of ceasing exercise (19,22,29,46). By contrast, the increase in CD was maintained after detraining and represents one mechanism underlying the sustained increase in M/I. This is consistent with the finding that CD is maintained even after 12 weeks of detraining in endurance-trained subjects (30). While this study does not account for all possible mechanisms affecting exercise-induced increases in M/I, of the measured factors, CD was the only measured variable to remain higher after detraining (Fig. 6).

Our results show that short-lasting adaptations to exercise, including but not limited to increases in GLUT4, AMPKα1, and GS activation, account for approximately one-fourth of the exercise training–induced improvement in M/I (i.e., the 7% improvement in M/I that dissipated with detraining vs. the 18% improvement that was maintained) and that other long-lasting mechanisms are responsible for the remaining proportion of the improvement in M/I. The results of the regression analysis suggest that as much as one-half of the 18% increase in M/I that remains
after detraining could be attributable to increases in CD ($r^2 = 0.49$), whereas the remaining half is likely due to other chronic adaptations not addressed in this study. This is consistent with reports that 45–65% of the resistance to insulin-stimulated glucose uptake is a result of limitations in the delivery of glucose to muscle (42). The contribution of CD to M/I is supported by experimental data from animal models in which either occlusion of capillaries (44) or deletion of vascular endothelial growth factor, causing low CD (47), reduces insulin-stimulated glucose uptake. Conversely, the AEX-induced increase in CD would increase the diffusible surface area to enhance glucose flux from blood to muscle. This is supported by a recent study assessing the effects of an experimental increase in capillarization on insulin sensitivity in a rodent model (48). Concordant with our findings, this group reported that increased capillarization (17–20%) in the absence of other metabolic adaptations (e.g., no effects on AMPK, GLUT4, pAkt, or GS activation) increased insulin sensitivity by 24%. The findings of our study, along with data from animal models, provide evidence that AEX-induced increases in skeletal muscle CD contribute to improvements in insulin sensitivity in older adults.

By measuring several intramuscular mechanisms known to enhance insulin sensitivity, the design of this study allowed us to identify a contribution of changes in CD to M/I. We acknowledge, however, that other mechanisms such as decreases in inflammatory cytokine expression or intramyocellular lipids, as well as improved glucose phosphorylation or mitochondrial adaptations in muscle, may also contribute to the sustained increase in insulin sensitivity. Also, the study design controlled for substantial changes in body weight, but we cannot exclude the possibility that the modest (2 kg) weight loss and decrease in body fat contributed to increases in M/I. Improvements in blood flow (49) and insulin-mediated recruitment of capillaries (rev. in 50) may also contribute to exercise-induced improvements in M/I. While this study is unable to distinguish the independent effects of CD per se from improvements in blood flow and recruitment of capillaries, it is clear that having higher CD in skeletal muscle provides for greater diffusion of glucose.

While the sample size is a potential limitation of this study, a strength of the study is measurement of M/I using a hyperinsulinemic-euglycemic clamp, with muscle sampling immediately before and during the clamp for measurement of CD and intramuscular mechanisms that affect insulin-stimulated glucose uptake, all within the same experiment. Another strength is the selection of older men and women in good health but with lower CD than their younger counterparts (1,2) and risk factors for cardiovascular disease and diabetes, including a sedentary lifestyle. AEX training in older adults can increase CD to levels similar to those in younger subjects (2,16); therefore, this represents one mechanism by which age-associated declines in insulin sensitivity could be ameliorated. The study included subjects with a range of glucose tolerance levels, but subjects with type 2 diabetes were excluded to avoid confounding effects of medications and comorbid diseases on study outcomes. Future studies will need to confirm these findings in subjects with type 2 diabetes and other diseases associated with impaired glucose metabolism.

In conclusion, this integrative, translational investigation demonstrates that AEX training increases skeletal muscle CD in older adults and that the increase in CD contributes to the sustained improvement in glucose metabolism independent of several intramuscular glucose transport mechanisms. If sustained, this increase in CD has the potential to mitigate and possibly prevent declines in glucose metabolism in susceptible older adults and potentially reduce progression to impaired glucose tolerance and type 2 diabetes.

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**Author Contributions.** S.J.P. wrote the manuscript. S.J.P., A.P.G., and A.S.R. conceived of and designed the research. S.J.P., A.P.G., H.K.O., E.R.C., D.C., J.B.B., and A.S.R. collected the data, performed the experiments, analyzed the data, and revised the manuscript. S.J.P. 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.
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References
1. Ryan NA, Zwetsloot KA, Westerkamp LM, Hickner RC, Polfah WE, Gavin TP. Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. J Appl Physiol (1985) 2006;100:178–185
2. Coggan AR, Spinu RJ, King DS, et al. Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. J Gerontol 1992;47:B71–B76
3. Prior SJ, McKenzie MJ, Joseph LJ, et al. Reduced skeletal muscle capillarization and glucose intolerance. Microcirculation 2009;16:203–212
4. Solomon TP, Haus JM, Li Y, Kirwan JP. Progressive hyperglycemia across the glucose tolerance continuum in older obese adults is related to skeletal muscle capillarization and nitric oxide bioavailability. J Clin Endocrinol Metab 2011;96:1377–1384
5. Lilloja S, Young AA, Culter CL, et al. Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. J Clin Invest 1987;80:415–424
6. Pinkney JH, Stenhoeve CD, Coppack SW, Yudkin JS. Endothelial dysfunction: cause of the insulin resistance syndrome. Diabetes 1997;46(Suppl 2):S9–S13
7. Regitnig W, Ellmerer M, Fauler G, et al. Assessment of transcapillary glucose exchange in human skeletal muscle and adipose tissue. Am J Physiol Endocrinol Metab 2003;285:E241–E251
8. Herkner H, Klein N, Joukhadar C, et al. Transcapillary insulin transfer in human skeletal muscle. Eur J Clin Invest 2003;33:141–146
9. Prior SJ, Joseph LJ, Brandauer J, Katzle LI, Hagberg JM, Ryan AS. Reduction in mid thigh low-density muscle with aerobic exercise training and weight loss impacts glucose tolerance in older men. J Clin Endocrinol Metab 2007;92:880–886
10. Dangel DR, Pratley RE, Hagberg JM, Rogus EM, Goldberg AP. Distinct effects of aerobic exercise training and weight loss on glucose homeostasis in obese sedentary men. J Appl Physiol (1985) 1996;81:318–325
11. Hughes VA, Fiatarone MA, Fielding RA, et al. Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance. Am J Physiol 1993;264:E862–E866
12. Dela F, Mikines KJ, Larsen JJ, Galbo H. Training-induced enhancement of insulin action in human skeletal muscle: the influence of aging. J Gerontol A Biol Sci Med Sci 1996;51:B247
13. Ferrara CM, Goldberg AP, Ortmeyer HK, Ryan AS. Effects of aerobic and resistive exercise training on glucose disposal and skeletal muscle metabolism in older men. J Gerontol A Biol Sci Med Sci 2002;57:403–410
14. Kowlew WC, Barrett-Conner E, Fowler SE, et al.; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403
15. Tuomilehto J, Lindström J, Eriksson JJ, et al.; Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343–1350
16. Coggan AR, Spinu RJ, King DS, et al. Skeletal muscle adaptations to endurance training in 60- to 70-year-old men and women. J Appl Physiol (1985) 1992;72:1780–1786
17. Charles M, Charfi N, Verney J, et al. Effect of endurance training on muscle microvascular filtration capacity and vascular bed morphometry in the elderly. Acta Physiol (Oxf) 2006;187:399–406
18. Prior SJ, Blumenthal JB, Katzle LI, Goldberg AP, Ryan AS. Increased skeletal muscle capillarization after aerobic exercise training and weight loss improves insulin sensitivity in adults with IGT. Diabetes Care 2014;37:1469–1475
19. Yukovich MD, Arciero PJ, Kuhrt WM, Racette SB, Hansen PA, Holloszy JO. Changes in insulin action and GLUT-4 with 6 days of inactivity in endurance runners. J Appl Physiol (1985) 1996;80:240–244
20. Neuffer PD, Shinebarger MH, Dohm GL. Effect of training and detraining on skeletal muscle glucose transporter (GLUT4) content in rats. Can J Physiol Pharmacol 1992;70:1286–1290
21. Reynolds TH 4th, Brozinick JT Jr, Larkin LM, Cushman SW. Transient enhancement of GLUT-4 levels in rat epitrochlealis muscle after exercise training. J Appl Physiol (1985) 2000;88:2240–2245
22. McCoy M, Proietto J, Hargreaves M. Effect of detraining on GLUT-4 protein in human skeletal muscle. J Appl Physiol (1985) 1994;77:1532–1536
23. Dela F, Ploug T, Handberg A, et al. Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM. Diabetes 1994;43:862–865
24. Frasig C, Jørgensen SB, Hardie DG, Richter EA, Wojtaszewski JF. 5′-AMP-activated protein kinase activity and protein expression are regulated by endurance training in human skeletal muscle. Am J Physiol Endocrinol Metab 2004;286:E411–E417
25. Nielsen JN, Mustard KJ, Graham DA, et al. 5′-AMP-activated protein kinase activity and subunit expression in exercise-trained human skeletal muscle. J Appl Physiol (1985) 2003;94:631–641
26. Christ-Roberts CY, Pratapanawat T, Pratapanawat W, et al. Exercise training increases glycoen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. Metabolism 2004;53:1233–1242
27. Ryan AS, Katzle LI, Prior SJ, McLenithan JC, Goldberg AP, Ortney HK. Aerobic exercise plus weight loss improves insulin sensitivity and increases skeletal muscle glycoen synthase activity in older men. J Gerontol A Biol Sci Med Sci 2014;69:790–798
28. Ryan AS, Ortney HK, Sorkin JD. Exercise with calorie restriction improves insulin sensitivity and glycoen synthase activity in obese postmenopausal women with impaired glucose tolerance. Am J Physiol Endocrinol Metab 2012;302:E145–E152
29. Mikines KJ, Sonne B, Trorier N, Galbo H. Effects of acute exercise and detraining on insulin action in trained men. J Appl Physiol (1985) 1989;66:704–711
30. Coyle EF, Martin WH 3rd, Pettey SF, Coleman D. Manual feedback technique for the control of blood glucose concentration. In Scovia NB et al., Eds. Automation in Analytical Chemistry (Technician Symposium). New York, Mediad, 1966, p. 489–491
31. Houston ME, Bentzen H, Larsen H. Interrelationships between skeletal muscle adaptations and performance as studied by detraining and retraining. Acta Physiol Scand 1979;105:163–170
32. Karonen M, Kentala E, Mustala O. The effects of training on heart rate; a longitudinal study. Ann Med Exp Biol Fenn 1957;35:489–491
33. Andersen R, Swedlow RS, Pfeitzey S, Coleman D. Manual feedback technique for the control of blood glucose concentration. In Scovin NB et al., Eds. Automation in Analytical Chemistry (Technician Symposium). New York, Mediad, 1966, p. 489–491
34. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223
35. Hennessey JV, Chramiak JA, Della Ventura S, Guertin J, MacLean DB. Incr ease in percutaneous muscle biopsy yield with a suction-enhancement technique. J Appl Physiol (1985) 1997;82:1739–1742
36. Porter MM, Koolage CW, Lexell J. Biopsy sampling requirements for the estimation of muscle capillarization. Muscle Nerve 2002;26:546–548
37. Danson MJ, Hough DW. Citrate synthase from hyperthermophilic Archaea. Methods Enzymol 2001;331:3–12
38. Joseph LJ, Prigoe RL, Blumenthal JB, Ryan AS, Goldberg AP. Weight loss and low-intensity exercise for the treatment of metabolic syndrome in obese postmenopausal women. J Gerontol A Biol Sci Med Sci 2011;66:1022–1029
39. Hounard JA, Horthagay T, Johns RA, et al. Effect of short-term training cessation on performance measures in distance runners. Int J Sports Med 1992;13:572–576
40. Frasig C, Rose AJ, Trebbak JT, Kiens B, Richter EA, Wojtaszewski JF. Effects of endurance exercise training on insulin signaling in human skeletal muscle: interactions at the level of phosphatidylinositol 3-kinase, Akt, and AS160. Diabetes 2007;56:2093–2102
41. Consitt LA, Van Meter J, Newton CA, et al. Impairments in site-specific AS160 phosphorylation and effects of exercise training. Diabetes 2013;62:3437–3447.
42. Wasserman DH. Four grams of glucose. Am J Physiol Endocrinol Metab 2009;296:E11–E21.
43. Sjöstrand M, Gudbjörnsdottir S, Strindberg L, Lönnroth P. Delayed transcapillary delivery of insulin to muscle interstitial fluid after oral glucose load in obese subjects [retracted in: Diabetes 2005;54:2266]. Diabetes 2005;54:152–157.
44. Vollus GC, Bradley EA, Roberts MK, et al. Graded occlusion of perfused rat muscle vasculature decreases insulin action. Clin Sci (Lond) 2007;112:457–466.
45. Fell RD, Terblanche SE, Ivy JL, Young JC, Holloszy JO. Effect of muscle glycogen content on glucose uptake following exercise. J Appl Physiol 1982;52:434–437.
46. Mujika I, Padilla S. Muscular characteristics of detraining in humans. Med Sci Sports Exerc 2001;33:1297–1303.
47. Bonner JS, Lantier L, Hasenour CM, James FD, Bracy DP, Wasserman DH. Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. Diabetes 2013;62:572–580.
48. Akerstrom T, Laub L, Vedel K, et al. Increased skeletal muscle capillarization enhances insulin sensitivity. Am J Physiol Endocrinol Metab 2014;307:E1105–E1116.
49. De Filippis E, Cusi K, Ocampo G, et al. Exercise-induced improvement in vasodilatory function accompanies increased insulin sensitivity in obesity and type 2 diabetes mellitus. J Clin Endocrinol Metab 2006;91:4903–4910.
50. Rattigan S, Bussey CT, Ross RM, Richards SM. Obesity, insulin resistance, and capillary recruitment. Microcirculation 2007;14:299–309.

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