Increased Skeletal Muscle Capillarization After Aerobic Exercise Training and Weight Loss Improves Insulin Sensitivity in Adults With IGT

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OBJECTIVE

Transcapillary transport of insulin is one determinant of glucose uptake by skeletal muscle; thus, a reduction in capillary density (CD) may worsen insulin sensitivity. Skeletal muscle CD is lower in older adults with impaired glucose tolerance (IGT) compared with those with normal glucose tolerance and may be modifiable through aerobic exercise training and weight loss (AEX+WL). We tested the hypothesis that 6-month AEX+WL would increase CD to improve insulin sensitivity and glucose tolerance in older adults with IGT.

RESEARCH DESIGN AND METHODS

Sixteen sedentary, overweight-obese (BMI 27–35 kg/m^2), older (63 ± 2 years) men and women with IGT underwent hyperinsulinemic-euglycemic clamps to measure insulin sensitivity, oral glucose tolerance tests, exercise and body composition testing, and vastus lateralis muscle biopsies to determine CD before and after 6-month AEX+WL.

RESULTS

Insulin sensitivity (M) and 120-min postprandial glucose (G_{120}) correlated with CD at baseline (r = 0.58 and r = −0.60, respectively, $P < 0.05$). AEX+WL increased maximal oxygen consumption (VO_{2max}) 18% ($P = 0.02$) and reduced weight and fat mass 8% ($P < 0.02$). CD increased 15% (264 ± 11 vs. 304 ± 14 capillaries/mm^2, $P = 0.01$), M increased 21% (42.4 ± 4.0 vs. 51.4 ± 4.3 μmol/kg FFM/min, $P < 0.05$), and G_{120} decreased 16% (9.35 ± 0.5 vs. 7.85 ± 0.5 mmol/L, $P = 0.008$) after AEX+WL. Regression analyses showed that the AEX+WL-induced increase in CD independently predicted the increase in M (r = 0.74, $P < 0.01$) as well as the decrease in G_{120} (r = −0.55, $P < 0.05$).

CONCLUSIONS

Six-month AEX+WL increases skeletal muscle CD in older adults with IGT. This represents one mechanism by which AEX+WL improves insulin sensitivity in older adults with IGT.

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More than 26% of older Americans have impaired glucose tolerance (IGT) (1), increasing their risk for developing type 2 diabetes. A sedentary lifestyle may further increase this risk through changes in skeletal muscle morphology and insulin signaling that worsen insulin resistance. We (2,3) and others (4,5) report low skeletal muscle capillarization in sedentary, insulin-resistant subjects that is inversely associated with the degree of glucose tolerance (2) and directly associated with insulin sensitivity (4,5) in cross-sectional studies.

As the major interface between the circulation and skeletal muscle, the microvasculature affects a number of physiological processes, including insulin resistance. Transcapillary transport of insulin is an important determinant of glucose uptake in skeletal muscle (6) and is a rate-limiting step for insulin action (7,8). The reduced capillary surface area in IGT and type 2 diabetes is associated with lower glucose uptake during insulin infusion (9); therefore, low skeletal muscle capillarization, along with defects in insulin signaling, can contribute to insulin resistance in IGT and type 2 diabetes by decreasing the available surface area for diffusion of insulin and glucose (10,11).

Lifestyle interventions including aerobic exercise and weight loss (AEX+WL) are a cornerstone of the treatment of insulin resistance and can prevent progression from IGT to type 2 diabetes in older people (12,13). AEX training increases skeletal muscle capillarization in healthy adults (14–18), but less is known about its effects on skeletal muscle capillarization in insulin-resistant subjects. One study showed that AEX training increases skeletal muscle capillarization in subjects with IGT (19) and such an increase in capillarization after AEX+WL may represent a mechanism for enhancing insulin and glucose delivery to skeletal muscle to improve insulin sensitivity. We hypothesized that a 6-month AEX+WL program would increase skeletal muscle capillarization in older adults with IGT, and that the increase in capillarization would translate to improvements in insulin sensitivity and glucose tolerance. To test this hypothesis, we examined skeletal muscle capillarization, glucose tolerance, and insulin sensitivity during hyperinsulinemic-euglycemic clamps before and after 6 months of AEX+WL in overweight-obese, older adults with IGT.

RESEARCH DESIGN AND METHODS

Subjects

Men and postmenopausal women 50–80 years of age, who were nonsmokers and had no previous diagnosis of diabetes or cardiovascular disease, were recruited from the Baltimore, MD, regional area to participate in studies examining metabolic responses to AEX+WL. Data from eight men and eight women (mean age 63 $\pm$ 2 years) with IGT and skeletal muscle samples for assessment of capillarization are reported herein; subject characteristics and certain metabolic data from a larger sample of the subjects were previously reported (20,21). Subjects were all weight stable ($<$2.0 kg weight change in past year), sedentary ($<$20 min of aerobic exercise two times per week), and screened by medical history questionnaire, physical examination, and fasting blood profile. Subjects were screened for IGT by oral glucose tolerance tests (OGTTs) according to American Diabetes Association criteria (22). All subjects were nonsmokers and showed no evidence of cancer; liver, renal, or hematological disease; or other medical disorders. The women in the study had not menstruated for at least 1 year. Subjects taking medications for hypertension or dyslipidemia were included if medically stable and if medications were not known to affect glucose metabolism. The research protocols were approved by the institutional review board at the University of Maryland School of Medicine. All subjects provided written informed consent.

AEX+WL Intervention

Prior to metabolic testing, subjects received 6–8 weeks of instruction on the Therapeutic Lifestyle Changes diet (23) in order to minimize potential confounding by changes in dietary composition during the intervention. Subjects followed the dietary guidelines and were weight stable for at least 2 weeks prior to baseline testing. After baseline testing, subjects were instructed to maintain the diet, and all subjects attended weekly weight loss classes for 6 months led by a registered dietitian. Individuals were counseled to restrict their caloric intake by 300–500 kcal/day to achieve $>$5% weight loss during the intervention. Compliance was monitored by 7-day food records using the American Diabetes Association exchange list system. In addition, all subjects underwent 6 months of supervised AEX training on treadmills at the Baltimore Veterans Affairs Medical Center Geriatric Research, Education, and Clinical Center exercise facility. Exercise intensity was prescribed by target heart rate range calculated using the Karvonen formula (24); heart rates were monitored during exercise with chest-strap heart rate monitors (Polar Electro Inc., Lake Success, NY). AEX training began at a volume of three sessions per week of 20 min at 50% of heart rate reserve, and gradually increased to three sessions per week of 45 min at ~85% of heart rate reserve, a level maintained for $>$4 months. Each exercise session included 5-min warm-up and cool-down phases. Compliance with the AEX+WL sessions was $>$85%.

Body Composition

Fat mass, fat-free mass, and percent body fat were measured with dual-energy X-ray absorptiometry (Prodigy; LUNAR Radiation Corp., Madison, WI). Intra-abdominal (IAF) and subcutaneous abdominal (SAF) fat areas were determined by a computed tomography scan at L_4-L_5 region using a Siemens Somatom Sensation 64 Scanner (Fairfield, CT) and Medical Image Processing, Analysis and Visualization software (MIPAV v.7.0.0; NIH, Bethesda, MD).

Maximal Oxygen Consumption

Maximal oxygen consumption (VO_{2max}) was measured by indirect calorimetry during a graded treadmill exercise test on a motorized treadmill. Subjects walked at a constant velocity throughout the protocol; grade was initially set to 0% and increased every 2 min thereafter to maximal effort. VO_{2max} was defined as the highest oxygen consumption value obtained for a full 30-s increment. Attainment of VO_{2max} was verified by standard physiological criteria (respiratory exchange ratio $>$1.10 or a plateau in VO_2 with an increase in workload).

OGTT

Subjects underwent 2-h OGTTs after a 12-h overnight fast. A catheter was placed in an antecubital vein and blood samples were drawn before and 30, 60, 90, and 120 min after the ingestion of a

75-g glucose solution. Blood samples were centrifuged and plasma was separated and stored at –80°C until analysis. Plasma glucose levels were analyzed with a glucose analyzer (2300 STAT Plus; YSI, Yellow Springs, OH). Plasma insulin levels were determined by radioimmunoassay (Millipore, St. Charles, MO). Glucose (G_{AUC}) and insulin (I_{AUC}) areas under the curve during the OGTT were calculated using the trapezoidal method. The homeostatic model assessment for insulin resistance was calculated as described by Matthews et al. (25).

Hyperinsulinemic-Euglycemic Clamp

Insulin-stimulated glucose uptake (M) was measured as an index of insulin sensitivity. Subjects were provided with all meals for the 2 days preceding the clamp to control nutrient intake. After a 12-h overnight fast, subjects underwent the hyperinsulinemic-euglycemic glucose clamp (26,27) as performed in our laboratory (20). Insulin was infused at a rate of 555 pmol/m^2/min, and M is reported in micromoles of glucose infused per kilogram of fat-free mass per minute (μmol/kg FFM/min). Clamp data were not available for one subject after AEX+WL due to a technical problem. Plasma glucose levels were analyzed at 5-min intervals using the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin levels were determined by radioimmunoassay (Millipore, St. Charles, MO). Mean insulin and glucose levels during the clamp were 1,131 ± 32 pmol/L and 5.1 ± 0.1 mmol/L, respectively, and did not differ before and after AEX+WL ($P > 0.4$).

Muscle Biopsies

Percutaneous needle biopsies were obtained from the vastus lateralis, ~12–13 cm above the patella on the anterolateral aspect of the right thigh using a Bergstrom needle (Stille, Solna, Sweden) as previously described (28). Muscle samples were rapidly embedded in optimal cutting temperature-tragacanth gum mixture, frozen, and stored at −80°C for histochemical analyses.

Capillary Density

Muscle was sectioned to a thickness of 14 μm on a cryostat and capillaries were identified using a modified double-stain technique (29). In brief, muscle sections were fixed in acetone and washed with 1% BSA. Sections were then incubated with primary antibodies (Ulex europaeus agglutinin I for endothelial cells and mouse anticollagen IV for muscle fiber perimeters) and secondary antibodies (rabbit anti-Ulex europaeus, goat anti-rabbit, and goat anti-mouse). Sections were then reacted with avidin-biotinylated alkaline phosphatase (ABC/AP; Dako, Carpinteria, CA) and the New Fuchsin Substrate System (Dako, Carpinteria, CA). Immunostained muscle sections were viewed under a light microscope and digital images were obtained (Eclipse Ti; Nikon Instruments Inc., Melville, NY). Quantification of capillarization was performed on at least 50 fibers for each sample (mean = 69 ± 4 fibers/sample); sampling a larger number of fibers does not improve the estimation of capillarization in human muscle (30). Images were analyzed using NIS Elements software (Nikon Instruments Inc.). The following four indices of capillarization were measured: *1*) capillary contacts (the number of capillaries in contact with each muscle fiber), *2*) individual capillary-to-fiber ratio (the number of whole capillary equivalents in contact with each muscle fiber), *3*) capillary density (CD; the number of capillaries per mm^2 of muscle cross-sectional area), and *4*) capillary-to-fiber perimeter exchange index (the number of capillaries per mm of muscle fiber perimeter).

Skeletal Muscle Fiber Type

For each vastus lateralis sample, a serial section was obtained and fiber type was determined using a myosin ATPase technique. After an initial 5-min incubation in an acid solution (0.4% sodium acetate, 0.6% sodium barbital, and 0.04 N hydrochloric acid), the samples were incubated for 45 min in an ATP reaction solution (0.6% glycine, 0.6% calcium chloride, 0.4% sodium chloride, 0.3% sodium hydroxide, and 0.17% ATP). This was followed by a 3-min incubation in 1% calcium chloride, a 3-min incubation in 2% cobalt chloride, and a 1-min incubation in 0.2% ammonium sulfide. Stained sections viewed under a light microscope and digital images were obtained (Eclipse Ti).

Statistical Analyses

The primary study outcomes were skeletal muscle capillarization, M, fasting plasma glucose, plasma glucose response to an OGTT, and VO_{2max}. Secondary variables included fasting plasma insulin, plasma insulin response to an OGTT, and body composition. Data are presented as means ± SEM. Statistical analyses were performed using SPSS v12.0 (IBM, Armonk, NY). Repeated-measures ANOVA was used to test for differences in outcome variables after AEX+WL, with sex used as a covariate in all analyses. Regression analyses were used to test for associations between capillarization variables and other primary and secondary variables; multivariable regression accounting for age, sex, and/or baseline levels of primary outcome variables was conducted where indicated. A type I error rate of $\alpha = 0.05$ was selected, and two-tailed probabilities are reported for all analyses.

RESULTS

Subject Characteristics and Responses to AEX+WL

Subject characteristics, body composition, and cardiorespiratory fitness levels before and after 6-month AEX+WL are presented in Table 1. In response to the AEX+WL intervention, subjects reduced their body weight and fat mass by 8% and their BMI by 7% ($P < 0.05$ for all). The subjects did not have a statistically significant reduction in IAF, but did reduce SAF by 9% ($P = 0.007$). The subjects significantly increased VO_{2max} (L/min) by 18% ($P = 0.02$).

AEX+WL significantly increased skeletal muscle capillarization (Table 2), with a 15% increase in CD ($P = 0.01$), a 14% increase in capillary-to-fiber perimeter exchange index ($P = 0.004$), and a greater number of capillaries in contact with each skeletal muscle fiber (capillary contacts and capillary-to-fiber ratio, $P < 0.05$). The proportion of type I or II muscle fibers did not change after AEX+WL (65 ± 4 vs. 67 ± 5% type I fibers, $P = 0.75$) nor did skeletal muscle fiber area and perimeter (≤2% difference, $P > 0.68$). These results indicate that the increase in CD was due to an increase in the number of capillaries, not a reduction in muscle fiber size.

Data from glucose tolerance tests and hyperinsulinemic-euglycemic clamps are presented in Table 2. Fasting plasma glucose and insulin concentrations were numerically lower after AEX+WL, but the differences were not statistically

Table 1—Subject characteristics and responses to 6-month AEX+WL

	Baseline	AEX+WL	% Change	*P* value
Sex (male/female)	8/8	—	—	—
Race (white/black)	11/5	—	—	—
Age (years)	63 ± 2	—	—	—
Weight (kg)	91 ± 3	84 ± 3	**−8%**	**0.03**
BMI (kg/m^2)	32.0 ± 1	29.7 ± 1	**−7%**	**0.02**
Body fat (%)	39.2 ± 1.4	36.9 ± 1.7	**−2.3%**	**0.003**
Fat mass (kg)	34.8 ± 1.7	31.9 ± 2.1	**−8%**	**0.001**
Fat-free mass (kg)	55.0 ± 1.7	54.3 ± 1.7	−1%	0.16
IAF (cm^2)	158 ± 24	145 ± 22	−8%	0.41
SAF (cm^2)	422 ± 39	383 ± 44	**−9%**	**0.007**
VO_{2max} (mL/kg/min)	19.4 ± 1.2	24.0 ± 1.9	**+24%**	**0.003**
VO_{2max} (L/min)	1.75 ± 0.11	2.07 ± 0.18	**+18%**	**0.02**

Data are presented as means ± SEM adjusted for sex unless otherwise indicated. Statistically significant changes are shown in boldface.

significant. AEX+WL reduced 120-min postprandial glucose concentrations (G_{120}) by 16% (P = 0.008), reduced 120-min postprandial insulin concentrations by 31% (P = 0.04), and reduced G_{AUC} by 10% (P = 0.03). I_{AUC} was numerically lower after AEX+WL (P = 0.08). Likewise, AEX+WL increased M (μmol/kg FFM/min) by 21% (P = 0.04).

Relationships Between Metabolic Variables and Skeletal Muscle Capillarization

At baseline, M (μmol/kg FFM/min) directly correlated with CD (r = 0.62, P = 0.01) and tended to inversely correlate with IAF (r = −0.49, P = 0.07) in bivariate analyses. M was not associated with the proportion of type I or II muscle fibers ($r = |0.0–0.27|$, $P > 0.33$ for all). Baseline G_{120} correlated only with CD (r = −0.68, P = 0.004). In multivariable regression analyses accounting for age, sex, and body weight in the model, baseline CD was the only variable independently associated with M (partial r = 0.58, P = 0.04) (Fig. 1*A*) and G_{120} (partial r = −0.60, P = 0.03) (Fig. 1*B*). Inclusion of IAF as an independent variable slightly reduced the overall r values in the regression models but did not affect the independent relationships (partial r values) between CD and M or G_{120} in the models.

After AEX+WL, the change in M (μmol/kg FFM/min) directly correlated with the change in CD (r = 0.53, P = 0.04) but not with the changes in VO_{2max}, body weight, body fat, IAF, or SAF (r = −0.19 to 0.29, $P > 0.33$) in bivariate analyses. Likewise, the change in G_{120} inversely correlated with the change in CD (r = −0.51, P = 0.04). In multivariable regression analyses accounting for age, sex, and the change in body weight, the AEX+WL-induced increase in CD independently correlated with the increase in M (partial r = 0.74, P = 0.006) (Fig. 2*A*) and the decrease in G_{120} (partial r = −0.55, P = 0.04) (Fig. 2*B*).

CONCLUSIONS

Insulin resistance and diabetes are health problems that affect more than one-third of older adults. Although lifestyle interventions including exercise and weight loss can improve glucose tolerance and reduce risk for type 2 diabetes (12,13), many of the underlying mechanisms remain elusive. The current study shows that despite the capillary rarefaction found in the skeletal muscle of sedentary, older adults with IGT, AEX+WL significantly increases skeletal muscle capillarization. Furthermore, this is the first study to show that increases in capillarization are directly associated with improvements in glucose tolerance and insulin sensitivity measured during a hyperinsulinemic-euglycemic clamp to our knowledge. Therefore, despite impaired angiogenesis and capillary rarefaction in sedentary people with insulin resistance and IGT, the ability to increase capillarization in skeletal muscle is maintained and likely contributes to AEX+WL-induced improvements

Table 2—Skeletal muscle capillarization, glucose tolerance, and insulin sensitivity before and after 6-month AEX+WL

	Baseline	AEX+WL	% Change	*P* value
Skeletal muscle				
Capillary contacts (capillaries/fiber)	3.65 ± 0.14	4.00 ± 0.17	**+10%**	**0.03**
Capillary-to-fiber ratio (whole capillary equivalents/fiber)	1.30 ± 0.06	1.46 ± 0.07	**+12%**	**0.04**
Capillary-to-fiber perimeter exchange index (capillaries/mm)	4.36 ± 0.14	4.96 ± 0.16	**+14%**	**0.004**
CD (capillaries/mm^2)	264 ± 11	304 ± 14	**+15%**	**0.01**
Fiber area (μm^2)	5,334 ± 289	5,219 ± 281	−2%	0.68
Fiber perimeter (μm)	295 ± 8	293 ± 9	−1%	0.73
Glucose tolerance				
Fasting plasma glucose (mmol/L)	5.6 ± 0.2	5.4 ± 0.2	−3%	0.28
Fasting insulin (pmol/L)	103 ± 11	92 ± 10	−11%	0.29
120-min postprandial glucose (mmol/L)	9.35 ± 0.5	7.85 ± 0.5	**−16%**	**0.008**
120-min postprandial insulin (pmol/L)	839 ± 139	580 ± 111	**−31%**	**0.04**
G_{AUC} (mmol/L/120 min)	1,075 ± 46	967 ± 57	**−10%**	**0.03**
I_{AUC} (pmol/L/120 min)	73,162 ± 10,299	57,172 ± 7,689	−22%	0.08
Insulin sensitivity				
M (mg/kg/min)	4.49 ± 0.32	5.81 ± 0.46	**+29%**	**0.004**
M (μmol/kg FFM/min)	42.4 ± 4.0	51.4 ± 4.3	**+21%**	**0.04**

Data are presented as means ± SEM adjusted for sex unless otherwise indicated. Statistically significant changes are shown in boldface.

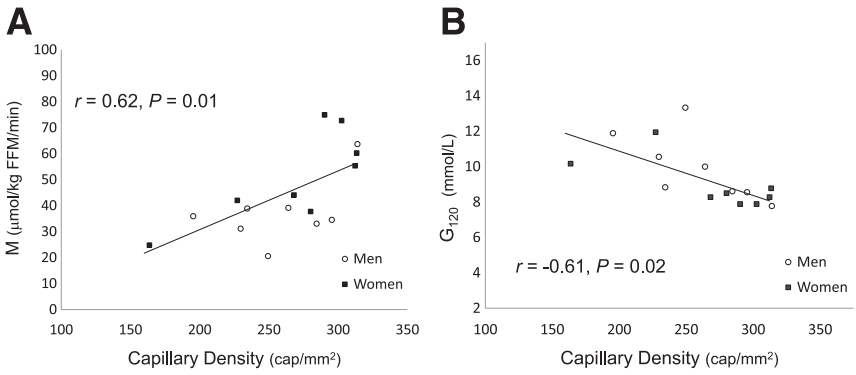

Figure 1—Scatterplots depicting the relationships between skeletal muscle CD and insulin sensitivity (M) (*A*) and 120-min postprandial glucose (G_{120}) (*B*) at baseline in overweight-obese, older adults with IGT. Bivariate correlation coefficients are shown in the figures; partial correlation coefficients from regression models are $r = 0.58$ and $r = -0.60$, respectively. $P < 0.05$.

in insulin sensitivity in older adults at risk for type 2 diabetes.

Skeletal muscle capillarization increases after AEX training in healthy young (14,18) and older (15,17) subjects; however, few studies have examined the effects of AEX training with or without WL on capillarization in subjects with IGT or type 2 diabetes to date. In one study, AEX training increased capillarization by 10% in a small group of men with IGT (19). Our results are concordant with these findings, indicating that skeletal muscle capillarization does increase with AEX+WL in both men and women with IGT. Another study using a combined AEX and strength training intervention showed an increase in the number of capillaries per fiber and in muscle fiber size, but no change in CD in subjects with type 2 diabetes (31). The maintenance of CD with increases in fiber size in that study suggests that angiogenesis occurred in skeletal muscle and that diabetes-associated capillary rarefaction may be reversible. As there were no changes in fiber size after AEX+WL in our study, increases in capillarization were consistent across the measurements we report. Weight loss independent of AEX may not be associated with an increase in skeletal muscle capillarization (32); however, one study showed increases in CD with large amounts (>20%) of weight loss (33). Additional study is required to distinguish the independent effects of AEX and WL on skeletal muscle capillarization in older subjects with IGT.

Previous cross-sectional studies from our group and others demonstrate relationships between skeletal muscle capillarization and insulin sensitivity or glucose tolerance (2–5). The longitudinal study design in the present report allowed us to test the effects of an AEX+WL intervention that targets skeletal muscle, and to examine its effects on skeletal muscle capillarization and changes in glucose metabolism. Concordant with previous studies (2–5), we find a direct relationship between capillarization and insulin sensitivity at baseline. Furthermore, our results show a strong relationship between AEX+WL-induced changes in capillarization and insulin sensitivity in people at high risk for type 2 diabetes. Although these data do not demonstrate true causality, data from animal models do demonstrate a direct role of capillarization in determining insulin sensitivity. For example, Vollus et al. (34) show that a graded occlusion of capillaries in a rodent model reduces insulin action and causes a stepwise reduction in insulin-stimulated glucose uptake in skeletal muscle ranging from 9 to 60%. Impairments in the recruitment and perfusion of capillaries by insulin also contribute to insulin resistance in humans and animals (see reference 35 for review); thus, it stands to reason that by having more capillaries in skeletal muscle, there is a larger capillary surface area that can be recruited and perfused in response to insulin. AEX training also improves endothelial vasoreactivity in subjects with IGT, which may play a role in improving insulin sensitivity through increased blood flow (36); however, the effects of capillarization and capillary recruitment appear to be independent of blood flow (11). Collectively, these findings support the conclusion that AEX+WL-induced increases in capillarization contribute to improvements in insulin sensitivity in older subjects with IGT.

In this study, subjects with IGT improved insulin sensitivity by >20% and improved glucose tolerance to near-normal levels (G_{120} = 7.85 mmol/L), with 9 of the 16 subjects reverting to normal glucose tolerance after 6 months of AEX+WL. Apart from skeletal muscle capillarization, other mechanisms including improvements in insulin signaling, gene and protein expression, GLUT4 content and translocation (see reference 37 for review), and body composition (38–40) may contribute to these metabolic improvements. We did not find a significant relationship

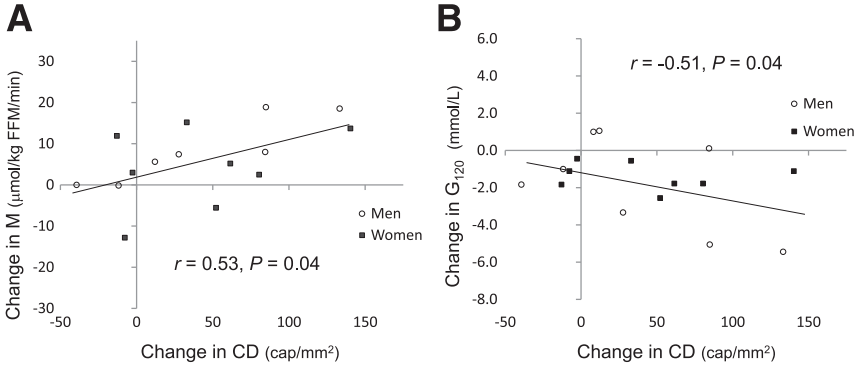

Figure 2—Scatterplots depicting the relationships between the AEX+WL-induced change in skeletal muscle CD and insulin sensitivity (M) (*A*) and 120-min postprandial glucose (G_{120}) (*B*) in overweight-obese older adults with IGT. Bivariate correlation coefficients are shown in the figures; partial correlation coefficients from regression models are $r = 0.74$, $P = 0.006$ and $r = -0.55$, $P = 0.04$, respectively.

between changes in insulin sensitivity and body weight or body composition in the current study, but our group previously showed that AEX+WL-induced increases in glycogen synthase activity are directly associated with improvements in insulin sensitivity (20). We did not assess these intramuscular mechanisms in the current study; thus, it is likely that increases in skeletal muscle capillarization work in tandem with these mechanisms to increase insulin action and improve insulin sensitivity in people with IGT. Studies are in progress to determine the relative contribution of capillarization and intramuscular mechanisms to AEX- and WL-induced improvements in insulin sensitivity.

In summary, these results show that skeletal muscle capillarization significantly increases after a 6-month AEX+WL intervention in previously sedentary older adults with IGT, and that there is a direct relationship between the increase in capillarization and the improvements in glucose tolerance and insulin sensitivity. These findings add further support to the concept that lifestyle interventions including AEX+WL will improve insulin sensitivity and reduce risk for type 2 diabetes, and indicate that increased skeletal muscle capillarization is one mechanism by which these metabolic improvements occur in overweight-obese, older adults with IGT.

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Author Contributions. S.J.P. conceived and designed the research, performed the experiments, analyzed the data, and wrote the manuscript. J.B.B. performed the experiments, analyzed the data, and edited and revised the manuscript. L.I.K. and A.P.G. conceived and designed the research and edited and revised the manuscript. A.S.R. conceived and designed the research, performed the experiments, analyzed the data, and edited 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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