Exercise Increases Intramyocellular Lipid and Insulin Sensitivity in Obese, Older, Insulin Resistant Adults: The Athlete's Paradox Revisited

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ABSTRACT

We previously reported an "athletes paradox" in the association between intramyocellular lipid (IMCL) and insulin resistance, in which endurance-trained athletes, who possess a high oxidative capacity and enhanced insulin sensitivity, also have higher IMCL. It is not clear, however, whether or not exercise training increases IMCL in insulin resistant subjects. The purpose of this study was to determine whether moderate exercise training would increase IMCL, oxidative capacity of muscle and insulin sensitivity in previously sedentary obese, insulin resistant older subjects. Twenty-five older (66.4±0.8 years) obese (BMI=30.3±0.7 kg/m^2) men (n=8) and women (n=17) completed a 16-week moderate but progressive exercise training program. Body weight and fat mass modestly but significantly (P<0.01) decreased. Insulin sensitivity, measured directly using the euglycemic-hyperinsulinemic clamp, was increased (21%, P=0.02) with modest improvements (7%, P=0.04) in aerobic fitness (VO_2max). Histochemical analyses of IMCL (Oil Red O staining), oxidative capacity (succinate dehydrogenase activity; SDH), glycogen content, capillary density and fiber type were performed on skeletal muscle biopsies. Exercise training increased IMCL (21%), SDH (19%), glycogen content (15%), capillary density (7%) and the percentage of type 1, slow-oxidative muscle fibers (from 50.8% to 55.7%), all $P\leq$0.05. In summary, independent of weight loss, chronic exercise in obese older adults induced several positive skeletal muscle adaptations, including increased substrate availability, enhanced oxidative capacity and improved insulin sensitivity. These results indicate that the metabolic inflexibility of skeletal muscle associated with aging and/or obesity can be largely restored with only moderate increases in physical activity.

Key Words: Insulin sensitivity, IMCL, Aging

INTRODUCTION

Several studies have demonstrated strong associations between high intramyocellular lipid (IMCL) content and skeletal muscle insulin resistance in obesity (23, 41), aging (9, 39, 42, 47) and type 2 diabetes (T2DM) (28, 33, 53). Yet, despite these numerous observations, we (21) described an "athletes paradox", which has since been confirmed by others (49, 53), in which highly insulin sensitive endurance trained athletes, have IMCL content similar to that observed in insulin resistant obese and T2DM subjects. We later reported that the exercise training-induced increase in IMCL was not limited to young lean highly trained athletes; in a group of older (~67 years) non-obese subjects, moderate aerobic exercise training increased IMCL content concomitant with improved oxidative capacity and overall fitness (43). Unfortunately, insulin sensitivity was not directly assessed in these subjects. Therefore, it is not clear whether exercise training enhances insulin sensitivity in conjunction with increases in IMCL in previously sedentary, overweight to obese, insulin resistant subjects.

Several studies have suggested that aging and obesity are similarly associated with insulin resistance (5, 10, 17, 20, 32), poor oxidative capacity (32, 34, 54), and a reduced capacity for substrate delivery, i.e. capillary number (19). However, it is not clear to what extent these negative attributes are caused by physical inactivity in aging and obesity. In young sedentary subjects exercise can clearly induce several changes in skeletal muscle that reflect an overall increase in metabolic flexibility, including increased insulin sensitivity (26), oxidative enzyme capacity (24, 36), capillary density (37) as well as an increase in glycogen storage (25, 40). It is uncertain whether similar adaptations can occur in subjects with a diminished metabolic flexibility, for example, older, obese subjects. Some studies have suggested that exercise improves the oxidative capacity of muscle but does not improve insulin sensitivity older men and

women (46). Others have reported improvements in insulin sensitivity (7, 13, 15) and enhanced oxidative capacity in older adults (46), although most of these studies have employed rigorous exercise regimes in non-obese, normal weight subjects. Taken together, previous studies suggest that exercise may improve some but perhaps not all aspects of metabolic inflexibility with aging and obesity, particularly in the context of moderate increases in physical activity. We sought to test the hypothesis that moderate exercise would increase IMCL in conjunction with improvements in insulin sensitivity in older, overweight to obese, insulin resistant adults, and that these positive adaptations would be part of an overall profile of enhanced metabolic flexibility within skeletal muscle.

METHODS

Study population. Men and women aged 60-75 years were recruited though print advertisements in the Pittsburgh area. Eligibility for inclusion included volunteers who were sedentary by self-reported (exercise ≤1-day per week), were weight stable (less than 3 kg weight-loss or gain in the previous 6 months), overweight to moderately obese (BMI 25.0-35.0 kg/m^2) and a non-smoker. Volunteers who passed the initial phone screen were further evaluated at the Clinical Translational Research Center. Uncontrolled hypertension (blood pressure >150 mmHg systolic and >95 mmHg diastolic), anemia (Hct<34%), elevated liver enzymes (25% above normal), proteinuria or hypothyroidism (sTSH>8 mIU/L) were considered exclusion criteria, as well as, chronic medications known to adversely affect glucose homeostasis. Further, if EKG abnormalities (i.e. tachycardia, uncontrolled arrhythmias, unstable ischemia) were observed during rest or graded exercise test, the subject was referred to their primary care physician for further evaluation.

Following the medical screen, volunteers completed a 2-h, 75-gram oral glucose tolerance test (OGTT) to determine glucose tolerance status. Volunteers with impaired fasting glucose (≥100 mg/dL), impaired glucose tolerance (IGT; 2-h OGTT glycemia >140 mg/dL but <200 mg/dL) and normal glucose tolerance (NGT; 2-h OGTT glycemia <140 mg/dL) were enrolled. All subjects gave written consent to the protocol approved by the University of Pittsburgh's Institutional Review Board.

Exercise intervention. Subjects were progressed to 4-5 days/week, 45-min/session (~180 min/week) of moderate (75% of heart rate max) intensity (determined by heart rate or perceived exertion (43)) supervised exercise (mostly walking and stationary cycling) for 16-weeks. After

the intervention, subjects were instructed on methods to maintain regular physical activity and encouraged to meet with the registered dietitian for nutrition counseling.

Insulin sensitivity. Rates of insulin-stimulated glucose disposal, considered the gold standard *in vivo* measure of insulin resistance (11), were assessed before and after the intervention using a hyperinsulinemic-euglycemic clamp (22, 45). Briefly, a continuous infusion of insulin (Humulin, Eli Lilly) was given at a rate of 40 $mU/m^2/min$ for 4-h and euglycemia (target=90 mg/dL) maintained using an adjustable infusion of 20% dextrose. Previous studies have demonstrated a near complete suppression of hepatic glucose output at this rate in subjects with this range of blood glucose values (50). Thus, the glucose infusion rate was assumed to represent a measure of skeletal muscle insulin-stimulated glucose disposal (2, 3). The glucose clamp was performed 48 hours following the last exercise session to avoid the potentially confounding effect of acute exercise on insulin sensitivity (12).

Body composition and maximal aerobic capacity (VO_2max). Total body fat and lean mass were assessed using DXA (Lunar, GE Lunar Prodigy and Encore 2005 software v9.30). As previously described (43), a VO_2max test was employed to determine both changes in physical fitness and the appropriate exercise intensity. Briefly, subjects performed a standard graded exercise test on a cycle ergometer until volitional exhaustion or one of the established criteria for VO_2max was reached (1). Heart rate, blood pressure and EKG were recorded prior to, during and immediately following this test.

Tissue analysis. Percutaneous biopsy samples were obtained in the fasted state on the mornings of the glucose clamp as previously described (16, 43). Following the excision, samples were cleared of any visible adipocytes using a standard dissecting microscope and blotted dry. Samples used for histochemistry were mounted on a small piece of cork with mounting medium,

placed into isopentane cooled with liquid nitrogen for 2-3 min and then into liquid nitrogen. All samples were stored at -70°C until analysis. Histochemical analyses were performed on serial sections using methods previously used in our laboratory (27, 28, 43). Samples from pre- and post-intervention were sectioned (10 μm) on a cryostat (Cryotome E, Shandon Scientific LTD) at -20°C and placed on individual precleaned glass slides. Slides representing 4-5 subjects were analyzed together to minimize staining bias. Each analysis included data from 150-300 fibers and intra-assay variability was less than 5%. Images were visualized using a Leica microscope (Leica DM 4000B, Leica Microsystems), digitally captured (Retiga 2000R camera, Q Imaging) and analyzed using specialized software (Northern Eclipse, v6.0, Empix Imaging). For analysis of intensity of staining (*i-iii* below), 4-5 images from both pre- and post-intervention sections were captured in 16-bit grayscale and averaged. *(i) Intramyocellular lipid content.* Triglyceride content was determined using Oil Red O staining as previously described (27). *(ii) Mitochondria activity.* Succinate dehydrogenase activity was measured using histochemical methods as previously described (43). *(iii) Glycogen content.* Skeletal muscle glycogen content was assessed using a standard Shiffs reagent protocol (28). (*iv*) *Capillary density.* Capillary density was determined using modified methods of Frisbee (18). Briefly, samples were allowed to air-dry of 15 min and then fixed for 1 h in 0.25% formaldehyde. Sections were incubated for 2 h with lectin (25μg/mL), rinsed and cover slips applied. Capillaries were visualized using a Tetramethyl Rhodamine Iso-Thiocyanate (TRITC) excitation filter. Capillary density was calculated as the total number of capillaries per total muscle area. *(v) Fiber type analysis.* The determination of Type 1, slow oxidative, and Type 2, fast glycolytic, skeletal muscle fiber types was determined using immnuohistochenistry. Briefly, antibodies specific for Type 1 and Type 2A fibers (Santa Cruz, Santa Cruz, CA) were applied using the manufacture's recommendations. Signals for

specific fibers were recorded using a flourescein isothiocyanate (FITC) excitation filter (Type 1) and a Tetramethyl Rhodamine Iso-Thiocyanate (TRITC) excitation filter (Type 2A). Type 2X fibers were assumed to be those that did not fluoresce with either filter. Approximately 100-300 total fibers were manually counted and relative fiber type percentage determined.

Statistical analysis. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Mac, v11). To address the effects of intervention on insulin sensitivity and skeletal muscle parameters, a paired t-test was applied to all data. Pearson's correlation analysis addressed the relation between changes in insulin sensitivity and tissue measures. The unequal numbers of males and females precluded a gender analysis owing to a lack of statistical power. Statistical significance was assumed *a priori* at $P<0.05$.

RESULTS

Study subjects, insulin sensitivity and aerobic fitness.

Thirty previously sedentary obese (BMI 30.3±0.7 kg/m^2) older (66.4±0.8 years) men (n=10) and women (n=20) were enrolled. Five subjects did not finish the intervention (drop out rate 16%); 4 due to time commitment conflicts and 1 to a newly diagnosed oncological disease. Only the 25 participants that completed the study were included in the analysis. Subjects were compliant with the exercise program as evidenced by achieving the recommended number of sessions per week (3.5±0.8; mean±SD). Moreover, additional data was collected to assess appropriate training intensities. Subjects expended an average of 833.3±78.6 kcals per week and 233.7±18.8 kcals per session. The exercise intensity was, on average 70.4±2.3% of VO_2max. Body weight and fat mass were modestly, but significantly (P<0.01) decreased (Table 1). Fat-free mass was unchanged by the aerobic training protocol. There was a fairly robust improvement (21%, P=0.02) in insulin sensitivity with intervention (Figure 1). Moderate aerobic training induced a modest improvement (7%, P=0.04) in maximal aerobic fitness (Table 1).

Skeletal muscle tissue analysis.

At baseline, no marker of skeletal muscle substrate availability or utilization was associated with insulin sensitivity. Figure 2A-E demonstrates the changes in skeletal muscle metabolism following exercise. Moderate aerobic exercise training significantly increased total IMCL content (21%, P<0.01), as measured by Oil Red O staining. Oxidative capacity, as measured by succinate dehydrogenase (SDH) activity, was significantly increased (22%, P<0.05) with exercise training. Glycogen was significantly increased (16%, P<0.05). Capillary density increased by 7% and the percentage of type 1, slow-oxidative fibers increased (13%, both P=0.05). However, none of the changes observed in IMCL or glycogen content, i.e. skeletal

muscle substrate availability or in capillary density or oxidative capacity, predicted the improvements in insulin sensitivity. Moreover, none of the adaptations in skeletal muscle metabolism predicted the improvements in aerobic fitness.

DISCUSSION.

Increased skeletal muscle intramyocellular lipid content (IMCL) has been associated with insulin resistance in human obesity (23, 41), aging (9, 39, 47) and type 2 diabetes mellitus (T2DM) (28, 33, 53). However, we have previously demonstrated an "athletes paradox" in which endurance-trained athletes have IMCL content similar to that observed in insulin resistant subjects (21). Moreover, there is evidence that physical inactivity has adverse effects on skeletal muscle metabolism, manifesting in poor oxidative capacity (29, 35), lower glycogen storage (8) and decreased capillary density (56). Therefore, we used a progressive, moderate-intensity aerobic training protocol to test the hypothesis that exercise training would induce positive adaptations in skeletal muscle substrate availability and utilization that would include increased IMCL content and enhanced insulin action. Our results clearly demonstrate that in obese, moderately insulin resistant, older adults, just modest increases in physical activity are required for increases in both IMCL and insulin sensitivity. In addition, this moderate exercise program induced other positive adaptations in skeletal muscle, including enhanced glycogen storage, oxidative capacity and capillary density in previously sedentary older adults. Therefore, many deleterious characteristics of skeletal muscle attributed to physical inactivity observed in aging and/or obesity can be largely restored with moderate exercise.

Insulin resistance is thought to be a key unifying feature of a myriad of conditions, including obesity and cardiovascular disease, and has been demonstrated to precede the development of type 2 diabetes mellitus. While exercise training clearly improves insulin sensitivity in younger (4) and middle-aged adults (14), the same may not be true for older populations (46). Here, we demonstrate that in a group of primarily obese previously sedentary older adults, moderate aerobic training induced a robust increase in insulin sensitivity

irrespective of baseline metabolic status. It is important to note that while our subjects lost a slight amount of fat-mass, these decreases are not as robust as those observed with diet-induced weight-loss (22). Therefore, the improvement in insulin sensitivity was primarily due to the increase in physical activity.

Physical inactivity is a key factor in the development of obesity, osteoporosis and sarcopenia observed in aging. Thus, the addition of regular exercise is often prescribed to ameliorate these conditions and other chronic diseases. Yet, despite our understanding of the overwhelming benefits of exercise, controversy still remains regarding the appropriate quantity of exercise required for metabolic improvement and in cardiorespiratory fitness. In agreement with our previous observation in older adults (43), relatively moderate-intensity aerobic training modestly improved fitness in this population. Moreover, these improvements are similar to those observed in younger adults with a similar exercise program (49).

The role of muscle triglycerides in insulin resistance is currently a topic of great interest. Higher amounts of IMCL as muscle triglycerides, while clearly associated with insulin resistance observed in obesity and type 2 diabetes, are also paradoxically observed in endurance-trained athletes. This study is the first to directly extend a paradoxical increase in both IMCL and insulin sensitivity to obese older adults with mild insulin resistance. In accord with these observations, we have previously observed an increase (~12%) in IMCL following exercise training in normal glucose tolerant non-obese older adults (43). Similar results have been observed in lean young adults (49). Importantly, we did not observe any relation between IMCL content and insulin sensitivity at baseline in this cohort. Therefore, it appears that IMCL as triglycerides do not confer insulin resistance, but rather the increases in IMCL content provide substrate for energy metabolism in the exercise-trained state (44, 52). This is also supported by the mounting

evidence, mostly in animal and cell culture models, that other lipid metabolites, such as diacylglycerol and/or ceramide, may be more directly linked to the development of insulin resistance (48, 51, 55).

It is well documented that obesity results in decreased postabsorptive rates of fatty acid metabolism despite similar rates of fatty acid uptake compared to lean controls (32). Moreover, rates of fatty acid oxidation are decreased with aging (46). Therefore, aging and obesity both contribute to a profile of metabolic inflexibility resulting in a greater reliance on skeletal muscle glycogen content for energy production. An important finding of the current study, in agreement with a previous report (30), was that moderate exercise training increased glycogen content probably due in part to an increased insulin sensitivity.

Mitochondrial defects have been observed in both obesity (29) and aging (31, 54) concomitant with impaired rates of substrate oxidation during rest and exercise. However, aerobic exercise increases the capacity of muscle for oxidation in non-obese young (6, 49) and older (43) individuals. Here we demonstrate that the oxidative capacity of muscle from obese, mildly insulin resistant older adults is increased following exercise training. Our previous finding of increased mitochondrial electron transport chain activity following exercise training in leaner older adults (38) supports these data. Moreover, the percentage of oxidative fibers was increased in agreement with previous reports (43). Taken together, the current data reflect a positive adaptation for increased skeletal muscle oxidative capacity with just moderate-intensity exercise.

The capacity for substrate or nutrient delivery was also increased as evidenced by an increase in capillary density following exercise training. Decreases in microvessel density have been reported in human (19) and animal (18) models of insulin resistance. This deleterious effect of nutrient oversupply and physical inactivity may indeed exacerbate the defects in oxidative

capacity by limiting the flux of substrates into and out of the cell. Thus, increasing capillary density *per se* may partially ameliorate perturbations in glucose and fatty acid homeostasis.

In summary, moderate increases in physical activity enhance insulin action and increases IMCL in older, overweight to obese, insulin resistant adults. These exercise-induced changes were found in the context of an overall improvement in metabolic flexibility evidenced by increased oxidative enzyme activity, a shift towards more oxidative fiber type profile and enhanced substrate delivery and storage capacity of muscle. However, the apparent disconnect between elevated lipid stores and enhanced insulin sensitivity remains to be elucidated. Nevertheless, this study indicates that physical inactivity plays a primary role in the development of insulin resistance and metabolic inflexibility in obesity and aging. Moreover, these data provide further support for the recommendation of lifestyle interventions, specifically aerobic exercise, for the treatment and prevention of insulin resistance.

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FIGURE LEGENDS

Figure 1. Effect of exercise on insulin sensitivity. Insulin sensitivity was determined using a hyperinsulinemic-euglycemic clamp prior to (PRE) and subsequent to (POST) exercise training as described in "Methods", n=25. Glucose infusion rate (GIR) is expressed relative to fat free mass (FFM). Statistical significance is indicated. Data are mean ± SEM.

Figure 2. Effects of exercise on substrate availability and capacity for oxidation. Histochemical analyses were performed on skeletal muscle biopsy samples obtained as described in "Methods", n=25. *Panel A*: Intramyocellular lipid content was measured by Oil Red O (ORO) staining. *Panel B*: Oxidative capacity was measured by succinate dehydrogenase (SDH) staining. *Panel C*: Glycogen content was measured by a Schiff's reagent protocol. *Panel D*: Capillary density. *Panel E*. Percentage of type 1, slow-oxidative fibers. Statistical significance is indicated. Data are mean ± SEM.

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Table 1. Subject characteristics and response to intervention.

	Pre	Post
N (male / female)	25 (8 / 17)	
Body weight (kg)	83.9 ± 2.0	82.6 ± 1.9*
BMI (kg/m^2)	30.3 ± 0.7	29.9 ± 0.7*
Fat mass (kg)	35.5 ± 1.5	33.8 ± 1.5*
Fat-free mass (kg)	45.2 ± 1.7	45.7 ± 1.7
VO_2max ($mL/kg_{FFM}/min$)	34.4 ± 6.7	36.7 ± 1.4*

Values are mean ± SEM. BMI, body mass index. FFM, Fat-free mass. VO_{2max}, maximal aerobic capacity.
*P<0.05, Significant Pre *versus* Post differences.

FIGURE 1

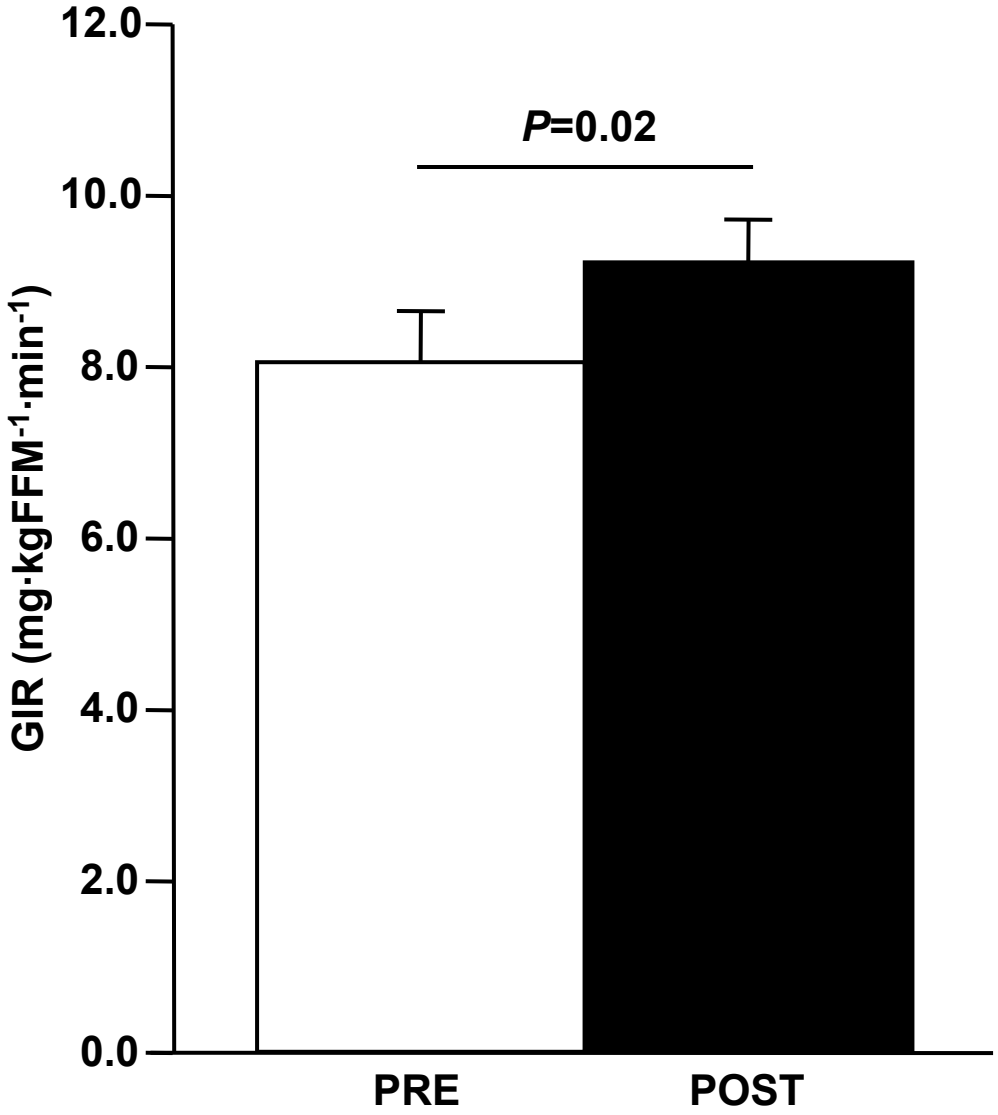
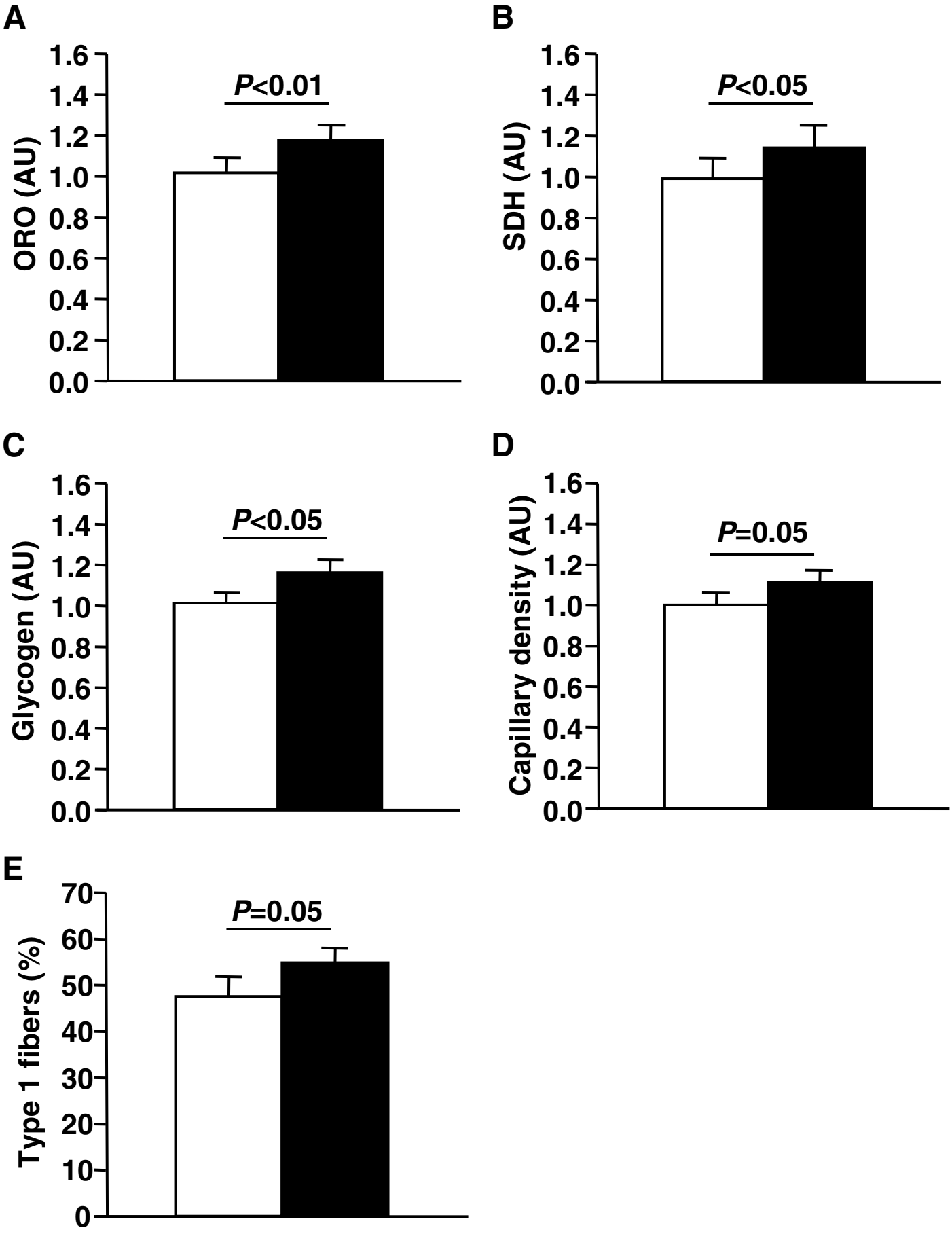


Figure 2


A
ORO (AU)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
P<0.01
B
SDH (AU)
P<0.05
C
Glycogen (AU)
P<0.05
D
Capillary density (AU)
P=0.05
E
Type 1 fibers (%)
70
60
50
40
30
20
10
0
P=0.05