Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisited

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Abstract

We previously reported an "athlete's paradox" in which endurance-trained athletes, who possess a high oxidative capacity and enhanced insulin sensitivity, also have higher intramyocellular lipid (IMCL) content. 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 overweight to obese, insulin-resistant, older subjects. Twenty-five older (66.4 ± 0.8 yr) obese (BMI = 30.3 ± 0.7 kg/m^2) men ($n = 9$) and women ($n = 16$) completed a 16-wk moderate but progressive exercise training program. Body weight and fat mass modestly but significantly ($P < 0.01$) decreased. Insulin sensitivity, measured using the euglycemic hyperinsulinemic clamp, was increased (21%, $P = 0.02$), with modest improvements (7%, $P = 0.04$) in aerobic fitness ($V_{O_{2peak}}$). 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 by 21%. In contrast, diacylglycerol and ceramide, measured by mass spectroscopy, were decreased ($n = 13$; −29% and −24%, respectively, $P < 0.05$) with exercise training. SDH (19%), glycogen content (15%), capillary density (7%), and the percentage of type I slow oxidative fibers (from 50.8 to 55.7%), all P 0.05, were increased after exercise. In summary, these results extend the athlete's paradox by demonstrating that chronic exercise in overweight to obese older adults improves insulin sensitivity in conjunction with favorable alterations in lipid partitioning and an enhanced oxidative capacity within muscle. Therefore, several key deleterious effects of aging and/or obesity on the metabolic profile of skeletal muscle can be reversed with only moderate increases in physical activity.

Keywords

insulin sensitivity; aging; diacylglycerol; ceramide

Several studies have demonstrated strong associations between high intramyocellular lipid (IMCL) content and skeletal muscle insulin resistance in obesity (25, 44), aging (11, 42, 45, 52), and type 2 diabetes (T2DM) (31, 36, 58). Yet, despite these numerous observations, we (23) described an "athlete's paradox" that has since been confirmed by others (54, 58) in which highly insulin-sensitive, endurance-trained athletes have IMCL content similar to that observed in insulin-resistant obese and T2DM subjects. We (46) later reported that the exercise training-induced increase in IMCL was not limited to young, lean, highly trained

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athletes; in a group of older (~67 yr), nonobese subjects, moderate aerobic exercise training increased IMCL content concomitant with improved oxidative capacity and overall fitness. 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 (7, 12, 19, 22, 35), poor oxidative capacity (35, 37, 59), and a reduced capacity for substrate delivery, i.e., capillary number (21). 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 (29), oxidative enzyme capacity (26, 39), and capillary density (40), as well as an increase in glycogen storage (27, 43). It is uncertain whether similar adaptations can occur in older, obese, insulin-resistant subjects. Some studies have suggested that exercise improves the oxidative capacity of muscle but does not improve insulin sensitivity older men and women (51). Others have reported improvements in insulin sensitivity (9, 15, 17) and enhanced oxidative capacity in older adults (51), although most of these studies have employed rigorous exercise regimes in nonobese, normal-weight subjects. 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 associated with an enhanced metabolic profile within skeletal muscle.

METHODS

Study Population

Men and women aged 60–75 yr were recruited though print advertisements in the Pittsburgh, PA, area. Eligibility for inclusion included volunteers who were sedentary by self-report (exercise   1 day/wk), weight stable (<3 kg weight loss or gain in the previous 6 mo), overweight to moderately obese [body mass index (BMI) 25.0–35.0 kg/m^2], and nonsmokers. 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 (sensitive TSH >8 mIU/l) were considered exclusion criteria, as well as chronic medications known to adversely affect glucose homeostasis. Furthermore, if EKG abnormalities (i.e., tachycardia, uncontrolled arrhythmias, unstable ischemia) were observed during rest or the 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-g oral glucose tolerance test (OGTT) to determine glucose tolerance status. Volunteers with impaired fasting glucose (100 mg/dl), impaired glucose tolerance (2-h OGTT glycemia >140 mg/dl, but <200 mg/dl), and normal glucose tolerance (2-h OGTT glycemia <140 mg/dl) were enrolled. All subjects gave written consent to the protocol, which was approved by the University of Pittsburgh's Institutional Review Board.

Exercise Intervention

Subjects were progressed to 4–5 days/wk, 45 min/session (~180 min/wk), of moderate-intensity [75% of heart rate maximum, determined by heart rate or perceived exertion (46)] supervised exercise (mostly walking and stationary cycling) for 16 wk. 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 measurement of insulin resistance (13), were assessed before and after the intervention using a hyperinsulinemic euglycemic clamp (24, 48). Briefly, a continuous infusion of insulin (Humulin; Eli Lilly) was given at a rate of 40 mU · m^2 · min^{-1} 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 (55). Thus, the glucose infusion rate was assumed to represent a measurement of skeletal muscle insulin-stimulated glucose disposal (2, 5). The glucose clamp was performed 48 h following the last exercise session to avoid the potentially confounding effect of acute exercise on insulin sensitivity (14).

Body Composition and Peak Aerobic Capacity

Total body fat and lean mass were assessed using dual-energy X-ray absorptiometry (GE Lunar Prodigy and Encore 2005 software v9.30). As previously described (46), a peak aerobic capacity ($V_{O_{2peak}}$) 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 $V_{O_{2peak}}$ 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 described previously (18, 46). Following the excision, samples were cleared of any visible adipocytes with a standard dissecting microscope and blotted dry. Portions of the tissue sample were snap-frozen in liquid nitrogen for biochemical analysis of lipid metabolites. Briefly, liquid nitrogen-frozen samples (~30 mg) were homogenized in ice-cold buffer (250 mM sucrose, 25 mM KCl, 50 mM Tris, and 0.5 mM EDTA, pH 7.4). Total diacylglycerol and ceramide content were measured by high-performance liquid chromatography-tandem mass spectrometry as previously described in detail (4).

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 placed 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 (30, 31, 46). Samples from pre- and postintervention were sectioned (10 μm) on a cryostat (Cryotome E; Shandon Scientific) at −20°C and placed on individual precleaned glass slides. Slides representing four to five subjects were analyzed together to minimize staining bias. Each analysis included data from 150–300 fibers, and intra-assay variability was <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 (see *Intramyocellular lipid content*, *Mitochondria activity*, and *Glycogen content* below), four to five images from both pre- and postintervention sections were captured in 16-bit grayscale and averaged.

Intramyocellular lipid content—Triglyceride content was determined using Oil Red O staining as described previously (30).

Mitochondria activity—Succinate dehydrogenase activity was measured using histochemical methods as described previously (46).

Glycogen content—Skeletal muscle glycogen content was assessed using a standard Shiffs reagent protocol (31).

Capillary density—Capillary density was determined using modified methods of Frisbee (20). Briefly, samples were allowed to air dry for 15 min and then fixed for 1 h in 0.25% formaldehyde. Sections were incubated for 2 h with lectin (25 μg/ml) and rinsed, and coverslips were applied. Capillaries were visualized using a tetramethylrhodamine isothiocyanate (TRITC) excitation filter. Capillary density was calculated as the total number of capillaries per total muscle area.

Fiber type analysis—The determination of type I slow oxidative and type II fast glycolytic skeletal muscle fiber types was determined using immnuohistochenistry. Briefly, antibodies specific for type I and type IIa fibers (Santa Cruz Biotechnology, Santa Cruz, CA) were applied using the manufacturer's recommendations. Signals for specific fibers were recorded using a flourescein isothiocyanate excitation filter (type I) and a TRITC excitation filter (type IIa). Type IIx 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 was 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 sex difference 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 overweight to obese (BMI 30.3 ± 0.7 kg/m^2) older (66.4 ± 0.8 yr) men ($n = 10$) and women ($n = 20$) were enrolled. Five subjects did not finish the intervention (dropout rate 16%), four due to time commitment conflicts and one to a newly diagnosed oncological disease. Only the 25 participants (men, $n = 9$; women, $n = 16$) that completed the study were included in the analysis. The subject compliance rate was $87.1 \pm 19.4\%$ (means $\pm$ SD, mean weekly sessions completed/recommended weekly sessions) for the exercise program, as evidenced by achieving the recommended number of sessions per week (3.5 ± 0.8, means $\pm$ SD). Moreover, additional data were collected to assess appropriate training intensities. Subjects expended an average of 833.3 ± 78.6 kcal/wk and 233.7 ± 18.8 kcal/session. The exercise intensity was, on average, $70.4 \pm 2.3\%$ of $V_{O_{2peak}}$. 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 (Fig. 1). When adjusted for changes in body weight and composition, the improvements in insulin sensitivity remained. Moderate aerobic training induced a modest improvement (7%, $P = 0.04$) in aerobic fitness (Table 1).

Skeletal Muscle Tissue Analysis

At baseline, no marker of skeletal muscle substrate availability or utilization was associated with insulin sensitivity. Additionally, baseline total body weight, BMI, and fat mass were unrelated to insulin sensitivity. Figure 2 demonstrates the changes in skeletal muscle substrate storage and capacity for oxidation following exercise training. 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 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 I slow oxidative fibers increased (11%, 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.

Skeletal muscle diacylglycerol and ceramide content were measured in a subset ($n = 13$) of men ($n = 4$) and women ($n = 9$) who completed the protocol due to limited sample size of the biopsies in some subjects. Their baseline characteristics and responses to intervention were similar to those for the large subject population (data not shown). Both total diacylglycerol ($P = 0.03$) and ceramide ($P = 0.01$) were decreased with exercise training (Fig. 3). The decrease in ceramide content was associated with the improvement in insulin sensitivity ($r^2 = 0.46$, $P < 0.01$). The decrease in diacylglycerol, however, was not associated with improvements in insulin sensitivity. In addition, changes in neither diacylglycerol nor ceramide were related to alterations in skeletal muscle substrate storage or capacity for oxidation.

DISCUSSION

Increased skeletal muscle IMCL lipid content has been associated with insulin resistance in human obesity (25, 44), aging (11, 42, 52), and T2DM (31, 36, 58). However, we (23) have previously demonstrated the athlete's paradox in which endurance-trained athletes have IMCL content similar to that observed in insulin-resistant subjects. Moreover, there is evidence that physical inactivity has adverse effects on skeletal muscle metabolism, manifesting in poor oxidative capacity (32, 38), lower glycogen storage (10), and decreased capillary density (61). 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.

The primary finding in this study was that just moderate increases in physical activity were sufficient to increase intramuscular triglycerides and decrease both diacylglycerol and ceramide content. These apparently favorable alterations in lipid partitioning within skeletal muscle were observed in conjunction with improved insulin sensitivity in these previously sedentary, insulin-resistant subjects. 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. Although exercise training clearly improves insulin sensitivity in younger (6) and middle-aged adults (16), the same may not be true for older populations

(51). 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, although our subjects lost a slight amount of fat mass, these decreases are not nearly as robust as those observed with diet-induced weight loss (24). Moreover, when adjusted for weight loss, improvements in insulin sensitivity were still present. Therefore, the improvement in insulin sensitivity was due primarily to the increase in physical activity.

Physical inactivity is a key factor in the development of obesity (37), osteoporosis (3), and sarcopenia (28) 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 (46), 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 (54).

The role of muscle triglycerides in insulin resistance is currently a topic of great interest. Higher amounts of IMCL as muscle triglycerides, although 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 insulin resistance. In accord with these observations, we have previously observed an increase (~12%) in IMCL following exercise training in normal, glucose-tolerant, nonobese older adults (46). Similar results have been observed in lean, young adults (54) and following acute exercise (50). Importantly, we did not observe any relation between IMCL content and insulin sensitivity at baseline in this cohort, possibly owing to the relatively homogeneous population investigated (23). Thus, IMCL as triglycerides per se may not confer insulin resistance, but rather, the increases in IMCL content provide substrate for energy metabolism in the exercise-trained state (47, 57). These findings are 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 (53, 56, 60).

Another important finding in our study was that both diacylglycerol and ceramide content are decreased with exercise training in conjunction with improved skeletal muscle insulin sensitivity. These results are in accordance with those of Bruce et al. (6), who found that exercise decreased these lipid metabolites along with enhanced glucose tolerance. However, although insulin sensitivity was not directly measured in their study, markers of enhanced fatty acid oxidation were observed. Thus, the decrease in lipid metabolite concentration following exercise may be related to an elevated oxidative capacity of muscle that may relate to improvements in insulin sensitivity. However, to date there is no direct evidence to support this mechanism. Although the baseline values of lipid metabolites in the current study were unrelated to insulin sensitivity, the alterations in skeletal muscle ceramide content did predict improvements in insulin-stimulated glucose uptake. However, these correlation data should be interpreted with caution given the homogeneous population and small sample size.

It has been reported (35) that obesity results in decreased postabsorptive rates of fatty acid metabolism despite similar rates of fatty acid uptake compared with lean controls. Moreover, rates of fatty acid oxidation are decreased with aging (51). Therefore, both aging and obesity 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 (33), 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 (32) and aging (34, 59) concomitant with impaired rates of substrate oxidation during rest and exercise. However, aerobic exercise increases the capacity of muscle for oxidation in nonobese young (8, 54) and older (46) individuals. Here we demonstrate that the markers of oxidative capacity of muscle from overweight to obese, mildly insulin-resistant older adults are increased following exercise training. Our previous finding of increased mitochondrial electron transport chain activity following exercise training in leaner, older adults (41) supports these data. Moreover, the percentage of oxidative fibers was increased, in agreement with previous reports (46, 49). It is still not clear, however, whether or not changes in fiber type or enhanced oxidative capacity cause alterations in muscle lipid levels or improved insulin sensitivity. Further studies, most likely to be conducted using animal models, should be performed to determine whether these contrasting changes in muscle triglyceride vs. diacylglycerol and ceramide are consistent in high-oxidative and low-oxidative muscles. 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 (21) and animal (20) 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, enhanced insulin action due to moderate increases in physical activity in older, overweight to obese, insulin-resistant adults is accompanied by a repartitioning of skeletal muscle lipid content demonstrated by increases in intramyocellular triglyceride but decreases in both diacylglycerol and ceramide. These exercise-induced changes were found in the context of an overall improvement in metabolic profile evidenced by increased oxidative enzyme activity, a shift toward more oxidative fiber type composition, and enhanced substrate delivery and storage capacity of muscle. This study suggests that physical inactivity plays a primary role in the development of insulin resistance in obesity and aging, perhaps through altered lipid partitioning within skeletal muscle and aberrant skeletal muscle metabolic profiles. Moreover, these data provide further support for the recommendation of lifestyle interventions, specifically aerobic exercise, for the treatment and prevention of insulin resistance.

Acknowledgments

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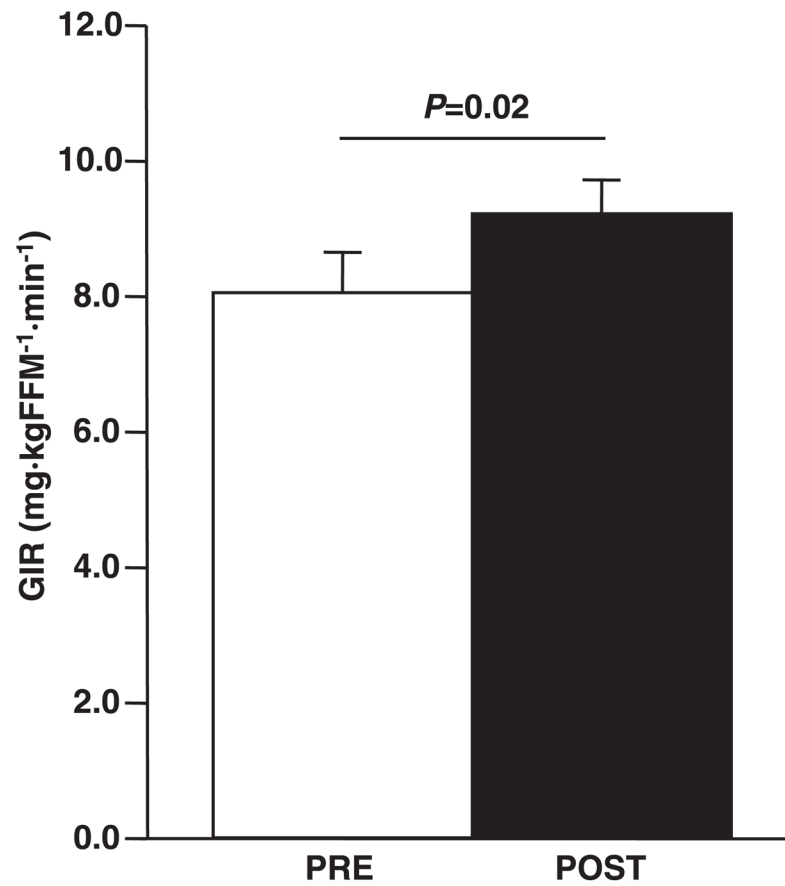
Fig. 1.
Effect of exercise on insulin sensitivity. Insulin sensitivity was determined using a hyperinsulinemic euglycemic clamp prior to and subsequent to exercise training, as described in METHODS; $n = 25$. Glucose infusion rate (GIR) is expressed relative to fat-free mass (FFM). Statistical significance is indicated. Data are means ± SE. PRE, preintervention; POST, postintervention.
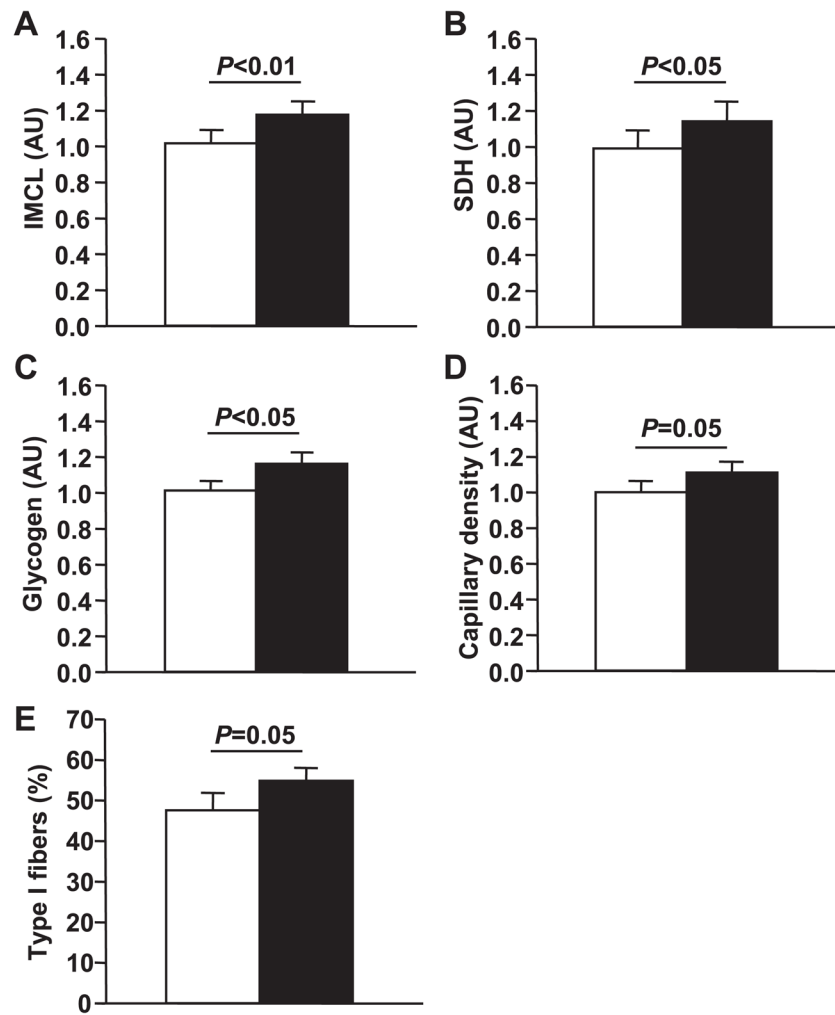
Fig. 2.
Effects of exercise on substrate availability and capacity for oxidation. Histochemical analyses were performed on skeletal muscle biopsy samples obtained as described in METHODS; $n = 25$. *A*: intramyocellular lipid (IMCL) content was measured by Oil Red O staining. *B*: oxidative capacity was measured by succinate dehydrogenase (SDH) staining. *C*: glycogen content was measured by a Schiff's reagent protocol. *D*: capillary density. *E*: percentage of type I slow oxidative fibers. Statistical significance is indicated. Data are means ± SE. AU, arbitrary units.
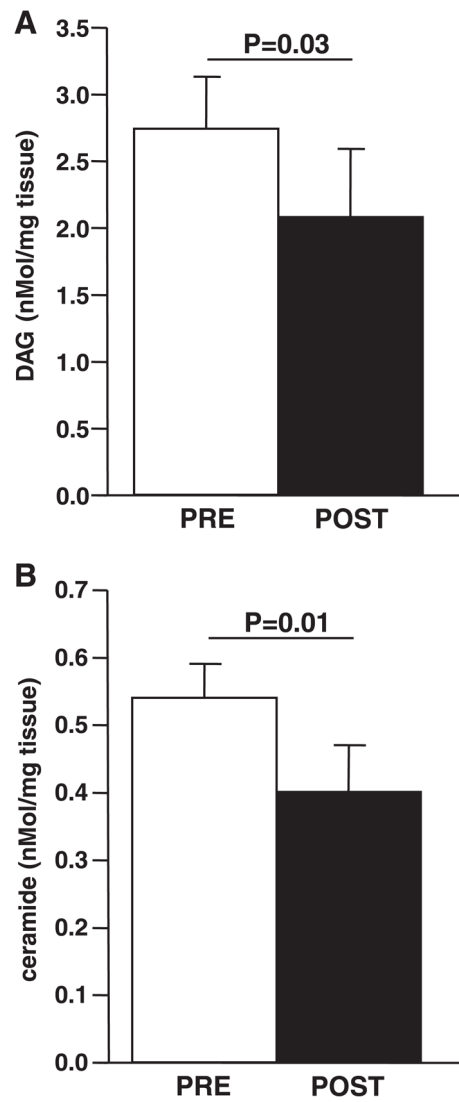
Fig. 3.
Effects of exercise on diacylglycerol (DAG) and ceramide content. DAG (*A*) and ceramide
(*B*) content were assessed from skeletal muscle biopsy samples as described in METHODS;
$n = 13$. Statistical significance is indicated. Data are means $\pm$ SE.

Table 1

Subject characteristics and response to intervention

	Pre	Post
n (Male/female)	25 (9/16)	
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[*]
FFM, kg	45.2±1.7	45.7±1.7
$V_{O_{2peak}}$, ml · kg FFM^{-1} · min^{-1}	34.4±6.7	36.7±1.4[*]

Values are means ± SE. Pre, preintervention; Post, postintervention; BMI, body mass index; FFM, fat-free mass; $V_{O_{2peak}}$, peak aerobic capacity.

[*] $P < 0.05$, significant Pre vs. Post differences.