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# Exercise training increases intramyocellular lipid and oxidative capacity in older adults

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**Pruchnic, Ryan, Andreas Katsiaras, Jing He, David E. Kelley, Carena Winters, and Bret H. Goodpaster.** Exercise training increases intramyocellular lipid and oxidative capacity in older adults. *Am J Physiol Endocrinol Metab* 287: E857–E862, 2004. First published June 29, 2004; doi:10.1152/ajpendo.00459.2003.—Intramyocellular lipid (IMCL) has been associated with insulin resistance. However, an association between IMCL and insulin resistance might be modulated by oxidative capacity in skeletal muscle. We examined the hypothesis that 12 wk of exercise training would increase both IMCL and the oxidative capacity of skeletal muscle in older (67.3 ± 0.7 yr), previously sedentary subjects ($n = 13$; 5 men and 8 women). Maximal aerobic capacity ($\dot{V}O_{2\,max}$) increased from 1.65 ± 0.20 to 1.85 ± 0.14 l/min ($P < 0.05$), and systemic fat oxidation induced by 1 h of cycle exercise at 45% of $\dot{V}O_{2\,max}$ increased ($P < 0.05$) from 15.03 ± 40 to 19.29 ± 0.80 ($\mu mol \cdot min^{-1} \cdot kg$ fat-free $mass^{-1}$). IMCL, determined by quantitative histological staining in vastus lateralis biopsies, increased ($P < 0.05$) from 22.9 ± 1.9 to 25.9 ± 2.6 arbitrary units (AU). The oxidative capacity of muscle, determined by succinate dehydrogenase staining intensity, significantly increased ($P < 0.05$) from 75.2 ± 5.2 to 83.9 ± 3.6 AU. The percentage of type I fibers significantly increased ($P < 0.05$) from 35.4 ± 2.1 to 40.1 ± 2.3%. In conclusion, exercise training increases IMCL in older persons in parallel with an enhanced capacity for fat oxidation.

skeletal muscle; physical activity; fiber type; triacylglycerol

ELEVATED INTRAMYOCELLULAR LIPID (IMCL), consisting primarily of triglyceride (TG), has been associated with insulin resistance (17, 40, 43, 46) and type 2 diabetes (18, 30) in middle-aged adults. It has recently been speculated that higher IMCL content is also linked to an impaired mitochondrial capacity for fatty acid oxidation within muscle (42). This is also consistent with reports that insulin-resistant skeletal muscle is characterized by lower oxidative capacity (50, 51) and lower postabsorptive rates of fatty acid oxidation (29). Other studies, however, suggest that muscle TGs are not invariably associated with insulin resistance. Endurance-trained athletes, who are markedly insulin sensitive, have similar IMCL to those with type 2 diabetes, suggesting that IMCL itself may not promote insulin resistance in the context of a high oxidative capacity in muscle (16). There have been few intervention-based human studies to examine whether exercise training might actually increase IMCL in populations at risk for the development of insulin resistance and type 2 diabetes.

Aging is also associated with metabolic dysregulation, including insulin resistance (8, 10), a higher prevalence of type 2 diabetes (21), and lower capacity for oxidative metabolism in muscle (4), although many of these defects can be attributed to age-related physical inactivity (5). Petersen et al. (41) recently reported that a reduced capacity for oxidative metabolism and higher IMCL are associated with insulin resistance of aging (41). Although this (41) and other (16, 26) cross-sectional studies highlight the interaction between IMCL and reduced oxidative capacity in insulin resistance, intervention-based studies are needed to further elucidate these associations. The effects of exercise training on IMCL content in the elderly, a population that is at a greater risk for metabolic disorders such as insulin resistance, have not been described. The purpose of this study was to determine the effects of exercise training on IMCL content, oxidative capacity in muscle, and the reliance on fat oxidation induced by exercise. We hypothesized that exercise training would increase IMCL in older (>65 yr) adults and that this would be concomitant with an increased oxidative capacity within muscle.

## METHODS

*Subjects.* Thirteen healthy older (67.3 ± 0.7 yr old) volunteers (8 women and 5 men) participated in this study after providing written informed consent. Before participation, all potential volunteers had a medical examination. None of the volunteers was currently engaged in regular (>1×/wk) exercise, nor had they gained or lost >2 kg body wt within the past 6 mo before the beginning of the study. None of the volunteers had type 2 diabetes. Individuals with coronary heart disease, peripheral vascular disease, or clinically significant hyperlipidemia (plasma TGs >3.95 mmol/l or total cholesterol levels >7.76 mmol/l) were excluded. Individuals with untreated hypertension were excluded. The protocol was approved by the University of Pittsburgh Institutional Review Board.

*Study protocol.* Before and after 12 wk of exercise, subjects had a percutaneous muscle biopsy, a test for maximal aerobic capacity ($\dot{V}O_{2\,max}$), and a 1-h submaximal exercise test to assess exercise-induced rates of substrate oxidation.

*Muscle biopsies.* Percutaneous biopsies were obtained in the General Clinical Research Center (GCRC) on a morning after an overnight fast. Subjects were given a standard 10 kcal/kg meal consisting of 50% carbohydrate, 30% fat, and 20% protein the night before the biopsy. Subjects were instructed not to perform physical exercise 48 h before the muscle biopsy procedure both before and after the training program to help prevent acute effects of exercise on muscle TG. Muscle biopsies were obtained from the middle region of the vastus lateralis (15 cm above the patella) and ~2 cm away from the fascia by percutaneous needle biopsy technique (11). Muscle specimens were trimmed, mounted, and frozen in isopentane cooled at −160°C by liquid nitrogen and stored at −80°C for histochemical analysis.

*Histochemical analysis.* Histochemical analyses were performed on light-microscopic micrographs of 8-μm-thick transverse cryostat sections at −29°C (HM505E; Microm, Walldorf, Germany). Initial sections from each frozen muscle block were inspected without stain to ensure that proper cross-sectional cuts were being obtained. Muscle

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sectioning, staining, and image analysis were performed by the same technician and done in a blinded manner with respect to subject. Pre- and postintervention muscle cryosections for each subject were placed on the same microscope slide so that histochemical staining and image analysis were performed under identical conditions for each subject, eliminating potentially confounding interassay variations in the treatment effect.

IMCLs were stained with the use of Oil Red O soluble dye, which stains neutral lipid (mainly TGs). This method has been described in detail previously (34). Briefly, after staining, a light microscope (Microphot-FXL; Nikon, Tokyo, Japan) was used to examine the stained muscle sections, using a ×40 oil immersion objective and bright field settings. Images were digitally captured using a charge-coupled device camera (Sony, Tokyo, Japan) and converted to gray-scale images. Contiguous fields of view within the biopsy section that were free from artifact were analyzed for lipid content; quantitative image analysis (Optima; Media Cybernetics, Silver Spring, MD) was then carried out on at least 80 fibers, or ~10 contiguous fibers/field. Oil Red staining was quantified as the difference in positive staining intensity from background. A control section treated with acetone and subsequently stained revealed no visible background staining.

Oxidative capacity of muscle was determined with succinate dehydrogenase (SDH) staining (9). A stock solution was made with 10 ml of 0.1 M PBS, 10 ml of 0.3% nitro blue tetrazolium, 4 ml of 0.065% KCN, 4 ml of 0.47% MgCl, and 8 ml of distilled water. The working solution was made by adding 2 ml of the stock solution to 200 μl of 1 M sodium succinate and 2–3 drops of 0.5% menadione. Slides were incubated for 45 min at room temperature and then washed in distilled water (3 times). The slides were then postfixed in 4% formaldehyde for 10 min, washed in distilled water, and mounted. Quantification of SDH staining was performed using image analysis of staining intensity, a method that has been verified with biochemical determination of SDH activity in muscle (36, 52). As negative control slides for this reaction, to assess background staining, sections were incubated in media without the enzyme substrate succinate.

The proportions of type I and type II muscle fibers were determined by both an anti-fast and an anti-slow myosin antibody staining method. After the blocking step, the slides were incubated with the primary antibody overnight at 4°C (mouse monoclonal antibody to myosin type II heavy chain; BioGenex, San Ramon, CA) and, in separate experiments, with a myosin type I heavy chain (mouse IgG hybridoma, obtained from Dr. James Sciote, Univ. of Pittsburgh). After several rinses in PBS, the slides were incubated with the secondary antibody, Alexa Fluor 488 goat anti-mouse IgG(H+L) (Molecular Probes, Eugene, OR), for 1 h. The sections were finally rinsed and gel mounted. All muscle fibers in the cryosection with and without anti-myosin staining were counted, and fiber type proportions were calculated accordingly.

*Maximal aerobic capacity.* Subjects performed a $\dot{V}_{O_2\,max}$ test on an electronically braked cycle ergometer (Ergoline 800S; SensorMedics, Yorba Linda, CA) to determine changes in physical fitness and to determine the target work rate (45% of $\dot{V}_{O_2\,max}$) used for the subsequent submaximal exercise studies. This test consisted of an initial warming-up period of 2 min with no-load pedaling. Then, depending on the individual, the graded exercise test began at 0–25 W for the first 2 min and then increased 10–25 W every 2 min thereafter until volitional exhaustion or one of the established criteria for $\dot{V}_{O_2\,max}$ had been reached (1). Heart rate, blood pressure, and ECG were recorded before, during, and immediately after this test. Subjects breathed through a mouthpiece connected to a two-way breathing valve (Hans Rudolph, Kansas City, MO) during the test, and expired air was collected via open-circuit spirometry (SensorMedics 2900) to determine oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$).

*Substrate oxidation during submaximal exercise.* At least 1 wk after the $\dot{V}_{O_2\,max}$ test, subjects were admitted to the GCRC the evening before the exercise study, where they were fed a standard dinner consisting of 10 kcal/kg of 50% carbohydrate-30% fat-20% protein and then fasted until completion of the exercise bout (~9:00 AM). This provided strict control of diet and physical activity 12 h before the acute submaximal exercise test. Additionally, they were instructed to avoid strenuous physical activity for 2 days before and to eat at least 200 g of carbohydrates for 3 days before the acute submaximal exercise test to ensure adequate glycogen stores for the exercise bout, since this may significantly influence substrate utilization during exercise (27). In addition, subjects were asked to record food intake in a diary for the 3 days before the exercise bout so that they could replicate their diet during the 3 days preceding the postexercise training bout of exercise. Nitrogen excretion rates were determined from two separate urine urea nitrogen measures performed at 12-h intervals to correct rates of lipid and carbohydrate oxidation for protein oxidation.

To measure substrate utilization during exercise, subjects cycled for 60 min on a bicycle ergometer (SensorMedics Ergoline 800S) at a work rate corresponding to 45% of their predetermined $\dot{V}_{O_2\,max}$. Indirect calorimetry was performed at 15, 30, 45, and 60 min of exercise to measure $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ to determine rates of total lipid and carbohydrate oxidation (12).

*Exercise training protocol.* Subjects participated in a 12-wk exercise training program consisting of cycling on a stationary bicycle or walking/jogging on an indoor treadmill. Subjects were required to perform a minimum of three supervised exercise sessions per week but were encouraged to perform one to two additional training sessions per week. For the first 4 wk, subjects were instructed to exercise for 30 min/session at an intensity within the range of 60–70% of maximal heart rate (corresponding to 50–60% $\dot{V}_{O_2\,max}$). During *weeks 5–8*, exercise sessions were increased to 40 min at the same intensity. During *weeks 9–12*, exercise sessions were continued at 40 min, but the intensity was increased to 70% of $\dot{V}_{O_2\,max}$.

Exercise intensity was quantified by the average heart rate recorded by a wireless monitor (Polar, Kempele, Finland) for each exercise session in the participant's personal exercise log. This provided an estimate of energy expenditure during each exercise bout based on the regression of heart rate and $\dot{V}_{O_2}$ determined at baseline and at *week 8* of the intervention.

*Statistical analysis.* Changes in IMCL, SDH staining, fiber type, $\dot{V}_{O_2\,max}$, and exercise-induced rates of fat oxidation were determined by paired *t*-tests. As a secondary objective, simple linear regression was used to examine whether the magnitudes of changes in any of these variables were correlated. The probability of detecting significant changes resulting from the intervention was set at an alpha level of $P = 0.05$.

## RESULTS

*Changes in physical fitness, generalized body composition, and markers of insulin resistance.* Before the intervention, subjects were not obese by body mass index (BMI) criteria and had normal fasting glucose values (Table 1). In addition, they were not severely hyperlipidemic, as evidenced by fasting serum triacylglycerol (143.1 ± 17.5 mg/dl) or total cholesterol (203.9 ± 7.2 mg/dl).

Subjects expended an average of 822 ± 77 kcal/wk during their exercise sessions over the course of the 12-wk intervention. The training program effectively enhanced physical fitness, as evidenced by an 11% improvement ($P < 0.05$) in $\dot{V}_{O_2\,max}$ (Table 2). Neither total nor the proportion of body fat changed, although there were slight reductions in fat free mass and total body weight (Table 1). Fasting glucose did not change, although there was a trend for both fasting insulin

Table 1. *Changes in physical fitness, generalized body composition, and markers of insulin resistance*

| | Pretraining | Posttraining |
|---|---|---|
| $\dot{V}O_{2\,max}$, l/min | 1.65±0.12 | 1.85±0.14* |
| Weight, kg | 76.9±2.6 | 76.0±2.6* |
| BMI, kg/m$^2$ | 28.0±1.0 | 27.6±1.0* |
| Fat mass, kg | 24.5±1.9 | 24.2±1.8 |
| Fat-free mass, kg | 52.4±2.2 | 51.8±2.2* |
| Body fat, % | 31.8±2.0 | 31.8±1.9 |
| Fasting blood glucose, mM | 5.11±0.09 | 5.10±0.09 |
| Fasting insulin, μU/ml | 11.72±1.77 | 10.49±1.47 |
| HOMA-IR | 2.69±0.43 | 2.38±0.34 |

Values are means ± SE; $n = 13$. $\dot{V}O_{2\,max}$, maximal oxygen consumption; BMI, body mass index; HOMA-IR, homeostatic assessment of insulin resistance. Fat mass and fat-free mass were determined by dual-energy X-ray absorptiometry (DEXA). *Significant change from pretraining ($P < 0.05$).

($P = 0.15$) and homeostatic model assessment for insulin resistance (HOMA-IR; $P = 0.14$) to change as markers of improved insulin resistance.

*Changes in exercise-induced fat oxidation.* Rates of systemic fat oxidation induced by submaximal exercise were measured before and after exercise training with gas-exchange indirect calorimetry. Although subjects performed submaximal exercise at the same absolute work rate before and after the exercise intervention, their $\dot{V}O_2$ was slightly lower (Table 2). As a result of their increased $\dot{V}O_{2\,max}$, subjects performed the posttraining submaximal exercise bout at lower relative exercise intensities, i.e., a percentage of $\dot{V}O_{2\,max}$ (Table 2).

Despite performing submaximal exercise at the same absolute work rate, average rates of fat oxidation during the 60-min bout of submaximal cycle exercise increased ($P < 0.05$) with training (Table 2). Consistent with this, there was a greater ($P < 0.05$) proportion of energy that was derived from fat after training (46 ± 3%) compared with baseline (65 ± 3%).

*Changes in IMCL and oxidative capacity.* Exercise training increased ($P < 0.05$) the content of IMCL, as determined by Oil Red O staining, by an average of 12.4% (Fig. 1). This increase in IMCL was consistent, occurring in 9 of 13 subjects. The increase in IMCL was not related to the IMCL content at baseline ($r^2 = 0.10$, $P = 0.29$). The oxidative capacity of muscle, determined by SDH staining intensity, also increased ($P < 0.05$) in 9 of 13 subjects by an average of 17.5% (Fig. 2). The magnitudes of the changes in IMCL and oxidative capacity were not correlated, nor were they related to the baseline degree of obesity, age, oxidative capacity, physical fitness, or IMCL content. Likewise, the IMCL and oxidative capacity response to training was not related to the magnitude of the changes in either $\dot{V}O_{2\,max}$ or fat oxidation induced by exercise.

Table 2. *Changes in exercise-induced fat oxidation*

| | Pretraining | Posttraining |
|---|---|---|
| Average power, W | 36±4 | 36±4 |
| Submaximal $\dot{V}O_2$, ml/min | 905±59 | 814±46* |
| Relative exercise intensity, %$\dot{V}O_{2\,max}$ | 55.7±2.08 | 45.2±2.03* |
| RER | 0.85±0.01 | 0.79±0.01* |
| Fat oxidation, μmol·min$^{-1}$·kg FFM$^{-1}$ | 15.02±1.16 | 18.55±0.81* |
| Fat oxidation, proportion of energy in % | 46±3 | 65±3* |

Values are means ± SE; $n = 13$. $\dot{V}O_2$, oxygen consumption; RER, respiratory exchange ratio; FFM, fat-free mass (determined by DEXA). Fat oxidation is expressed as an average rate during 60 min of exercise. *Significant change from preintervention ($P < 0.05$).

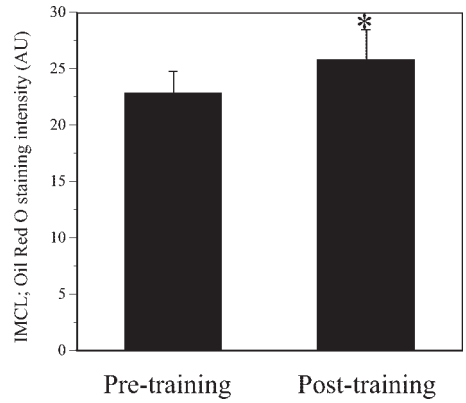


Fig. 1. Intramyocellular lipid (IMCL) content before and after a 12-wk exercise intervention. IMCL was determined by histochemical analysis of lipid droplets using Oil Red O staining (staining intensity quantified as arbitrary units; AU). Data are means + SE for the group ($n = 13$). *Significant change after intervention ($P < 0.05$).

The increase in oxidative capacity was also manifested by a significant change in muscle fiber type. The proportion of type I fibers determined by immunohistochemistry increased ($P < 0.05$) from 35.5 ± 2% at baseline to 40.1 ± 2% after training (Fig. 3). This was a consistent response; 11 of the 13 subjects had an increase in the proportion of type I fibers. It was possible that the anti-type II staining did not specifically stain type IIX fibers and that the number of type I fibers would be overestimated. We subsequently verified these results by performing an additional set of immunochemical experiments ($n = 8$) using an anti-type I myosin staining procedure. The proportion of type I fibers increased from 44 ± 5% to 57 ± 3% with training, which is consistent with the results calculated from anti-type II myosin (39 ± 5% to 48 ± 3% type I fibers for the same subjects). A total of 389 ± 41 fibers were counted at baseline, and 350 ± 42 total fibers were counted after the intervention.

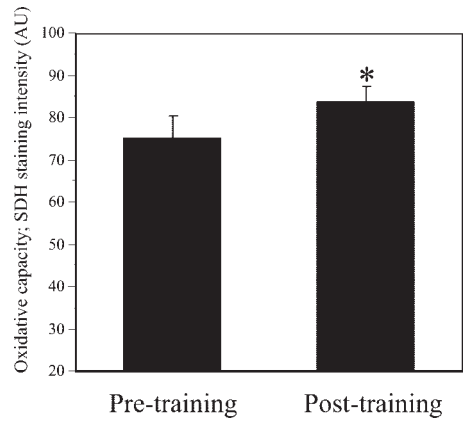


Fig. 2. Oxidative capacity in skeletal muscle before and after a 12-wk exercise training program, quantified by histochemical staining of succinate dehydrogenase (SDH) enzyme activity (staining intensity quantified as AU). Data are means + SE for the group ($n = 13$). *Significant change after intervention ($P < 0.05$).

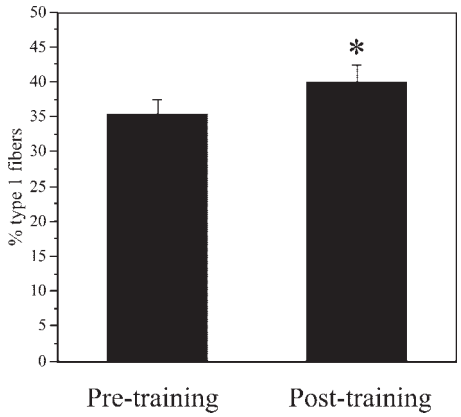


Fig. 3. Relative proportion of type I muscle fibers before and after a 12-wk exercise training program, quantified by anti-myosin immunohistochemistry. Data are means + SE for the group ($n = 13$). These data were calculated from a total of 389 ± 41 muscle fibers counted at baseline and 350 ± 42 total fibers counted after the intervention. *Significant change after intervention ($P < 0.05$).

## DISCUSSION

IMCL is linked with insulin resistance and type 2 diabetes, metabolic disorders that are highly prevalent in the elderly (15). However, the capacity for fatty acid oxidation may modulate the association between IMCL and insulin resistance. The key findings from this study were that exercise training in older men and women increased IMCL content and that this increase was observed in conjunction with an augmented oxidative capacity.

The increase in IMCL in these older subjects is consistent with our previous report of elevated IMCL in younger endurance-trained athletes (16) and with Bruce et al. (3), who concluded that the muscle oxidative capacity is a better predictor of insulin sensitivity than IMCL. Our results are also in accord with those of Schrauwen-Hinderling et al. (48), who found that increases in IMCL were an early response to training in younger adults. The current study extends these findings, indicating that the increase in IMCL with exercise training is not limited to intensive training, nor is it limited to younger adults. The effects of exercise training on IMCL are equivocal; some studies reported no effect on IMCL (23, 31), while others found that endurance training increased IMCL stores (16, 24, 48). It is possible that this discrepancy is partly due to the different methods used to assess IMCL. Generally, studies finding no effect of exercise on IMCL have relied on biochemical measures of IMCL (23, 31), which can be confounded by adipose tissue contamination of the biopsy specimen (19). In contrast, studies using NMR spectroscopy (48) or histological analysis (16) are able to clearly distinguish IMCL from any possible extracellular adipose tissue contamination. The magnitude of the change in IMCL in the current study (~12%) is less than the difference (68%) between younger endurance-trained athletes and their sedentary counterparts (16) or the ~40% increase reported for younger subjects by use of NMR spectroscopy (48). Although this is the first study to examine the effects of exercise training on IMCL specifically in older men and women, these results suggest that the magnitude of the response of older adults may be less than that observed for younger healthy subjects.

The increase in IMCL was observed in conjunction with an increased oxidative capacity of skeletal muscle and an enhanced ability to oxidize fatty acids during submaximal exercise, during which the majority of energy expenditure occurs within skeletal muscle. This exercise training-induced increase in fat oxidation is concordant with an augmented oxidative capacity of athletes (6, 13), who are markedly insulin sensitive (16). Although the primary aim of this study was not to directly measure training-induced changes in insulin resistance, there was a trend for fasting insulin and HOMA-IR as markers of insulin resistance to improve. Using the glucose clamp technique, we have demonstrated improvements in insulin sensitivity of previously sedentary subjects who completed a similar exercise training regime (14). Although exercise training-induced improvements in oxidative capacity in older adults have been observed without alterations in insulin sensitivity (49), most studies have reported improved insulin sensitivity of older men and women with exercise training (7, 25, 32).

Our results are also consistent with recent experiments performed in animal models indicating that IMCLs as TGs are not invariably detrimental to insulin action. Yu et al. (54) recently found that lipid infusion in animals induced skeletal muscle insulin resistance by elevation of fatty acyl-CoA and subsequent increases in diacylglycerol (DAG) but not TG within muscle tissue. Thus, according to these previous studies, it is likely that muscle TGs are merely a marker for other potentially harmful lipid metabolites such as ceramide and DAG in subjects with a poor capacity for fatty acid oxidation that have been shown to be directly implicated in skeletal muscle insulin resistance (28, 35, 38, 39, 47). It is also likely, however, that, in situations of reduced capacity for oxidative metabolism or in sedentary individuals in which there is lack of turnover of the muscle TG pool, that muscle TGs contribute to the source of ceramides, DAG, and hexosamines (47). The current study raises the intriguing hypothesis that the converse is true, i.e., that chronic exercise by some as-yet-undetermined mechanism increases muscle TGs while maintaining lower levels of these other potentially harmful metabolites. These studies would need to be performed by use of biochemical determination of lipid metabolites, since the histological approach of Oil Red O staining for IMCL includes quantification of both TGs and DAG.

Another primary finding in the current study was that exercise induced a shift in muscle fiber type toward more oxidative type I fibers, which have been shown to have higher IMCL content (2, 22). The literature on whether fiber type changes with endurance training is inconsistent. Lexell et al. (33) reported that despite evidence of small increases in type I fibers in endurance-trained rats after treadmill running, proportion of type I fibers is unaltered in endurance-trained humans, regardless of age. Tyni-Lenne et al. (53) suggested that the number of type I fibers may even decrease after endurance training. Conversely, studies by Hambrecht et al. (20) as well as Russell et al. (45), who also used an immunostaining method, support the current findings of an increase in type I fibers after exercise training. However, these studies did not address the effects of exercise on IMCL content. We did not determine the change in IMCL content within each fiber type, nor did we measure the change in muscle fiber size.

Although we found no gender differences in any of the responses to training, a limitation of our study was the lack of

sufficient statistical power to examine gender-specific responses to training. Further studies are needed to address whether there are gender differences in the training-induced increases in IMCL and oxidative capacity, as has been reported for gender differences in lipid metabolism during acute exercise (37, 44). Similarly, it would be important to determine whether varying levels of obesity, age, insulin resistance, IMCL content, and other factors may affect these observed responses to training.

In summary, a program of increased moderate exercise can increase the content of IMCL. This increase was observed concomitant with improvements in the oxidative capacity of skeletal muscle and an increase in oxidative type I myofibers caused by increased physical activity. This is the first such study examining changes in IMCL and oxidative capacity, specifically in older adults, a population at high risk for metabolic disorders and who may be particularly susceptible to the negative consequences of lipid-induced insulin resistance. Although the exact mechanisms are not yet fully elucidated, this provides further evidence that IMCL content per se is not necessarily linked to poor metabolic function in persons with an improved capacity for oxidative metabolism, including increases in mitochondria content and/or function.

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