Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity

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Tarnopolsky MA, Rennie CD, Robertshaw HA, Fedak-Tarnopolsky SN, Devries MC, Hamadeh MJ. Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. Am J Physiol Regul Integr Comp Physiol 292: R1271–R1278, 2007. First published November 9, 2006; doi:10.1152/ajpregu.00472.2006.—Impaired mitochondrial function and structure and intramyocellular lipid (IMCL) accumulation have been associated with obesity and Type 2 diabetes. We examined whether endurance exercise training and sex influenced IMCL and mitochondrial morphology using electron microscopy, whole-body substrate use, and mitochondrial enzyme activity. Untrained men (n = 5) and women (n = 7) were tested before and after 7 wk of endurance exercise training. Testing included 90 min of cycle ergometry at 60% $\dot{V}O_2$peak with preexercise muscle biopsies analyzed for IMCL and mitochondrial size/area using electron microscopy and short-chain β-hydroxyacyl-CoA dehydrogenase (SCHAD) and citrate synthase (CS) enzyme activity. Training increased the mean lipid area density (P = 0.090), the number of IMCL droplets (P = 0.055), the number of IMCL droplets in contact with mitochondria (P = 0.010), the total mitochondrial area (P < 0.001), and the size of individual mitochondrial fragments (P = 0.006). Women had higher mean lipid area density (P = 0.030) and number of IMCL droplets (P = 0.002) before and after training, but higher individual IMCL area only before training (P = 0.013), compared with men. Women oxidized more fat (P = 0.027) and less carbohydrate (P = 0.032) throughout the study. Training increased $\dot{V}O_2$peak (P = 0.001), %fat oxidation (P = 0.018), SCHAD activity (P = 0.003), and CS activity (P = 0.042). In summary, endurance exercise training increased IMCL area density due to an increase in the number of lipid droplets, whereas the increase in total mitochondrial area was due to an increase in the size of individual mitochondrial fragments. In addition, women have higher IMCL content compared with men due mainly to a greater number of individual droplets. Finally, endurance exercise training increased the proportion of IMCL in physical contact with mitochondria.

Intramuscular triglycerides; sex differences; lipid oxidation; mitochondria

ENDURANCE EXERCISE TRAINING in healthy men and women is often associated with an increase in the proportion of lipid oxidized during exercise (4, 22, 27, 34, 35), higher β-oxidation enzyme capacity (5, 33, 46), and an increase in intramyocellular lipid (IMCL) (15, 16, 33, 35). Endurance exercise training robustly increases mitochondrial enzyme capacity (5, 10, 16, 27, 33, 36) and total mitochondrial area/volume (13, 15, 16). This increase in mitochondrial oxidative capacity in endurance exercise-trained athletes likely explains the “athlete-obesity” IMCL paradox, for the high IMCL of athletes is accompanied by a greater oxidative capacity (9), which, hypothetically, could reduce the accumulation of metabolic byproducts associated with insulin resistance.

Lipid metabolism is also influenced by sex. Cross-sectional studies have shown that women oxidize proportionately more lipid compared with men during submaximal endurance exercise (4, 14, 27, 28, 32, 48). Irrespective of training status, women have a higher basal IMCL content and utilize more IMCL during acute exercise compared with men (39, 42). We (5, 27) and others (38) have found that neither mitochondrial enzyme capacity [i.e., citrate synthase (CS), complex I + III activity] nor β-oxidation enzyme activity are different between the sexes in spite of the higher IMCL content in women. However, IMCL accumulation in response to endurance exercise training does not seem to be affected, since in a cross-sectional study involving women and men of different training status, the basal IMCL content was similar within the same sex (42).

There are a number of methods used for IMCL determination, including biochemistry (15, 39, 42), proton magnetic resonance spectroscopy (1H-MRS) (11, 15, 45), histology (12, 47), and electron microscopy (EM) (13, 15, 16). With the use of either 1H-MRS or direct visualization of IMCL by EM, the values appear consistent between the two methods and equate to a range of 0.17% to 1.06% of the muscle area and volume (15). Unlike other techniques, the EM technique can also provide detailed data on IMCL and mitochondrial number and size, and their proximity and location (13, 15, 16). Given the interest in the role of IMCL and mitochondrial dysfunction in the development of obesity and Type 2 diabetes (T2DM) (17, 20) and the potential for exercise to be a countermeasure for these conditions (10, 36), a renewed interest in the role of IMCL and mitochondrial capacity in metabolism has emerged over the past few years (9, 11, 12, 19–21, 24, 29, 36, 37, 49).

In this study, we evaluated the independent and interactive effects of endurance exercise training and sex on IMCL/
mitochondrial morphology and β-oxidation/mitochondrial enzyme capacity in healthy young men and women. Our main objective was to use EM to directly visualize the morphology and the structural relationship between IMCL and mitochondria in response to 2 mo of endurance exercise training in both men and women.

METHODS

Subjects

Young (20–31 yr) men (n = 5) and women (n = 7) volunteered to participate in the study, which was approved by the McMaster Research Ethics Board and conformed to the principles of the Declaration of Helsinki. Subjects had not participated in physical activity more than twice per week (less than 1 h/session) for the preceding 6 mo and did not have any medical or orthopedic contraindications to physical activity. All women were eumenorrheic for at least 6 mo before the start of the study, and none experienced menstrual dysfunction during training. Five of the seven women were taking tricyclic oral contraceptives and did so for the study duration.

Initial Screening

A cycle ergometry test was performed to determine the peak oxygen consumption (V̇O₂peak) and to calculate the initial testing and training power outputs. V̇O₂peak was determined using a progressive exercise test on an electronically braked cycle ergometer and a computerized open-circuit gas collection system. V̇O₂peak was established when all three criteria were met: 1) the respiratory exchange ratio (RER) was higher than 1.12, 2) values for oxygen consumption reached a plateau or were the highest during the incremental ergometer protocol, and 3) pedal revolutions could not be maintained at or over 60 rpm despite vigorous encouragement. This was used to calculate the work intensity required to obtain 60% of the subject’s V̇O₂peak.

Body composition was assessed using dual energy X-ray absorptiometry (DEXA, QDR 1000W, Hologic, Waltham, MA) using whole body software for adults (V5.63). After the initial screening, women were matched with men according to V̇O₂peak/fat-free mass (FFM).

Experimental Design

Pre- and posttraining testing. The testing and training protocols were nearly identical to that previously reported by our laboratory (4, 27), with minor modifications. A 90-min ride at 60% V̇O₂peak was performed before (PRE) and after (POST) 7 wk of endurance exercise training. Each 90-min trial (PRE and POST) was performed at approximately the same time of day and under similar environmental conditions.

Two 4-day dietary records were collected 1 wk before the start and completion of the training and analyzed using computerized diet analysis software (Nutritionist Five, First Data Bank, San Bruno, CA) for the determination of total energy and %protein, carbohydrate, and fat intake (Table 1). Subjects were instructed to consume their habitual diet for 24 h before and to refrain from consuming caffeine and/or alcohol 12 h before the 90-min test rides. Subjects avoided exercise 48 h before testing. Women were tested during the early to midfollicular phase of their menstrual cycle, where the first day of menses or “bleeding” was considered the first day of the menstrual period.

All subjects arrived at the clinic between 0800 and 0930 after consuming a defined formula supplement (NutrBisics, Nestle Canada, Toronto, ON, Canada) 4 h before the scheduled start of exercise. The energy in the supplement was ~15% of the total daily energy intake (Table 1): 170 kJ (250 kcal) for women and 253 kJ (375 kcal) for men, with 59% carbohydrate, 16% fat, and 16% protein. Subjects were weighed, and a 20-gauge plastic catheter was inserted into the ante-cubital vein for blood sampling. A biopsy was taken from the vastus lateralis under local anesthesia before the commencement of exercise, as described previously (3). Visible fat and connective tissue were immediately trimmed (if necessary), and the samples were divided into two sections. Approximately 5 mg of muscle were immediately transferred to ice-cold 2% glutaraldehyde [buffered with 0.1 M sodium cacodylate (pH 7.4)] to preserve the muscle ultrastructure and was stored for less than 24 h at 4°C for EM analysis (see EM analysis). The remaining muscle sample was immediately frozen in liquid N₂ and stored at ~80°C. Following training, a muscle biopsy was taken from the same leg (randomly 5 cm proximal or distal to the first biopsy) at least 48 h after the final training bout.

For the test ride, subjects exercised on a cycle ergometer (Lode, Groningen, Netherlands) at 60% V̇O₂peak for 90 min before exercise training and at the same absolute exercise intensity following exercise training (same wattage). Respiratory measurements (V̇O₂, V̇CO₂, RER, and ventilation) and heart rate (HR) for both rides were recorded using a metabolic cart (Physio-Dyne, Fitness Instrument Technologies, Quogue, NY) at 0, 60, and 90 min of exercise. The values for the last 30 min of exercise were averaged. Venous blood was collected at 0 and 90 min of exercise for subsequent determination of glucose, lactate, glyceral, free fatty acids (FFA), total triglyceride, and insulin concentrations. Blood for the metabolites and hormone determination was collected in heparin- and nontreated-tubes, respectively. All samples were centrifuged at 1,200 g for 10 min, and the plasma and serum were aliquoted into 1.5 ml polyethylene microcentrifuge tubes and stored at ~80°C for subsequent analysis. During the ride, the subjects were allowed to consume water ad libitum with identical volumes consumed in all three testing sessions.

Exercise training. Subjects exercised on a cycle ergometer (Monark, Varberg, Sweden) for 1 h/session at 60% V̇O₂peak, 5 ± 1 days/wk for 7 wk. Training was monitored to ensure that subjects were cycling at the correct tension and speed. At 4 wk of training, V̇O₂peak was remeasured and the 60% training power output was recalculated and used for the remainder of the training program. Any missed sessions were recorded and were made up within the 7 wk of training.

Analytical Methods

Metabolites and hormones. Plasma glucose and lactate concentrations were determined using a glucose and lactate analyzer (Model 2300 Stat Plus, Yellow Springs Instrument, Yellow Springs, OH). Plasma FFA (NEFA C, Wako Pure Chemicals, Osaka, Japan), glyceral, and total triglyceride (Sigma Diagnostics, St. Louis, MO) concentrations were determined enzymatically using a spectrophotometric microplate reader (Bio-Rad, Benchmark, Hercules, CA). Insulin concentrations were determined using a commercially available radioimmunounassay kit (Coat-a-count, TKIN5, Diagnostic Products, Los Angeles, CA). For glucose, lactate, FFA, and insulin, the coefficients of variation (CV) were <10%. For glyceral and total triglycerides, the CVs were <15%.

Muscle Sample Analysis

Enzyme activity. CS enzyme activity was determined using 15–25 mg of wet muscle using a UV spectrophotometer (model 8453, Hewlett Packard, Wilmington, DE), as previously described by our group (5, 27). Samples were run in duplicate, and the intra-assay CV was 7 ± 5%. Short-chain β-hydroxy acyl-CoA dehydrogenase (SCHAD) activity was determined on a diluted homogenate after CS determination, using methods previously published by our group (5). Samples were run in duplicate, and the intra-assay CV was 7 ± 4%.

EM analysis. Muscle samples were fixed with 1% osmium tetroxide, dehydrated with ethanol, and embedded in Spurr’s resin. Thin sections (70 nm) were cut using an ultramicrotome (Ultracut E; Reichert, Vienna, Austria), placed on Cu/Pd grids (200 mesh size) and stained for 5 min in uranyl acetate followed by 2 min in lead acetate.
Sections were viewed at a magnification of ×6500 using a transmission electron microscope (TEM) (Jeol 1200 Ex; Jeol, Tokyo, Japan). Two micrographs from each of four different muscle fibers (i.e., a total of eight images per time point per person, 16 in total) were taken randomly and sequentially while blinded to sex and training status. The images were taken from a region containing only the intermyofibrillar region and another containing a portion of the subsarcolemmal region adjacent to the nucleus with most of this image containing intermyofibrillar area (Fig. 1). A total of 192 photographs was analyzed for the current study. The images were photographed using a 1-s exposure time and digitized using a white-light illuminator (C-80 Epi-Illumination UV Darkroom, Diamed, Mississauga, ON, Canada).

Lipid and mitochondrial analysis was determined using a computerized image analysis system (Image Pro Plus, ver. 4.0, Media Cybernetics, Silver Spring, MD). Criteria were set for lipid droplet and mitochondrial identification, and correct identification was confirmed by reviewing 12 random micrographs with a pathologist trained in EM (Dr. K. Chorneyko). Lipid droplets and mitochondrial fragments were circled and converted to actual size using a calibration grid. Values are reported as mean individual IMCL or mitochondrial size (µm²), total number of IMCL droplets, or mitochondria per square micrometer of tissue (#/µm² tissue⁻¹), and percentage IMCL or mitochondrial area “density” (mean size µm²/# IMCL-µm² tissue⁻¹, expressed as %area of muscle). This value is often reported as volume density using point counting volume estimates (13, 15, 16); however, given that standard TEM provides a two-dimensional image, we have referred to this as the percent of the muscle area that is occupied by IMCL or mitochondria. Furthermore, three-dimensional EM tomography has demonstrated that the mitochondria form a reticulum in skeletal muscle and are not distinct oval organelles (26, 30, 31); consequently, estimates of two-dimensional volume assuming that mitochondria are round or oval do not reflect the fact that each visible “mitochondrion” is actually a mitochondrial fragment. It is important to note that our data for both mitochondria and IMCL yield nearly identical percentage values to volumes reported using grid point counting estimates (15, 16).

To determine the number of EM micrographs required to obtain reproducible data, we had two trained observers sequentially add in the results of the IMCL and mitochondrial analysis from micrographs obtained from two different individuals (2 pictures/liver) and calculated the number of images required to obtain a plateau in the variance (i.e., further addition of images did not lower the variance). This value was between 6 and 8; consequently, we decided to use eight images per person per timepoint in the current study. With this method, the average (n = 2 observers) interassay CV for IMCL determination in the same subject was 4.7% for mean individual IMCL size and 6.1% for the number of IMCL droplets per area of muscle tissue. The corresponding value for IMCL content determined by 1H-MRS is ~ 6% (2). The test-retest CVs for the determination of the IMCL and mitochondria variables within the same subject separated by 48 h were <8% for all measured variables.

Calculations

Carbohydrate and fat oxidation was determined using indirect calorimetry (18). The nitrogen excretion rate was estimated to be 0.405 and 0.190 mg N₂·min⁻¹·kg⁻¹ body wt (PRE and POST rides) and 0.175 and 0.123 mg N₂·min⁻¹·kg⁻¹ body wt (PRE and POST rides) for men and women, respectively (44). The subjects were not fasted overnight; they had a light defined formula diet meal ~4.0 h before the samples were collected, and these were obtained at the same time pre- and postexercise for both sexes.

Statistical Analyses

The subject characteristics were analyzed using an unpaired t-test (age and height) or a two-way ANOVA (body weight, fat-free mass, %body fat, VO₂peak), with sex (men vs. women) as the between factor and time (PRE vs. POST) as the within factor (Version 5.0, Statistica, Tulsa, OK). The muscle biopsy (IMCL and mitochondria, SCHAD, and CS), dietary intake (energy, %fat, %CHO, and %protein), blood (insulin, glucose, triglycerides, glycerol, and free fatty acids), and metabolic (RER, %fat oxidation, and %CHO oxidation), data were analyzed using a two-way ANOVA with sex (men vs. women) as the between factor and time (PRE vs. POST) as the within factor. A Tukey’s post hoc analysis for unequal sample sizes was used to locate pair-wise differences when statistical significance was observed. We used a one-tailed test when analyzing the SCHAD and CS data, because we hypothesized a priori that training would increase muscle SCHAD and CS activity. We also used a one-tailed test when analyzing sex differences in IMCL area density, because we hypothesized a priori that women would have higher IMCL area density compared with men, based on data published by Roepstorff et al. (42). Given that there were no previous data on IMCL size or number, we could not make any a priori hypotheses regarding these two outcome variables. For all other analyses, we used two-tailed tests. Significance was set at P ≤ 0.05. Values are reported as means ± SD.
RESULTS

Descriptive Data

The descriptive characteristics of the subjects are given in Table 1. The men were taller ($P < 0.001$) and leaner ($P = 0.040$) than the women, with no sex difference in VO$_{2\text{peak}}$/body wt and VO$_{2\text{peak}}$/FFM. There were no changes in body weight or FFM following training. Training resulted in a 9% and 14% increase in VO$_{2\text{peak}}$ for men and women, respectively ($P < 0.001$). Plasma lactate concentration ($P < 0.001$) and HR ($P = 0.007$) were lower during exercise in the POST vs. PRE trial (data not shown). Total energy intake was higher in men compared with women ($P < 0.001$); however, there was no difference in the composition of the diet between the sexes, and training did not alter any of the dietary variables (Table 1).

RER and Energy Utilization

The RER during exercise was significantly lower for women compared with men during both trials (PRE and POST) ($P = 0.025$). Women oxidized proportionately more lipid ($P = 0.027$) and less CHO ($P = 0.032$) during exercise compared with men. Following training (POST), lipid oxidation was higher for both men and women compared with PRE ($P = 0.018$) (Table 1).

Muscle Sample Analysis

IMCL. IMCL values for women were significantly higher compared with men when expressed as the number of lipids per square micrometer of tissue (# IMCL·μm$^2$ tissue$^{-1}$, 43%, $P = 0.002$) and as mean IMCL area density (mean size # IMCL·μm$^2$ tissue$^{-1}$, 84%, $P = 0.030$); however, the difference in size of individual IMCL was present between men and women before training only, with women having 61% higher values than men ($P = 0.013$). Following training, there was a 42% increase in the number of IMCL droplets ($P = 0.055$) and a trend for an increase in mean IMCL area density (35%, $P = 0.090$), with no change in the mean size of individual IMCL droplets. Men and women showed a higher proportion of IMCL in contact with mitochondria following training (men, ↑ 21%; women, ↑ 12%; $P = 0.010$, Fig. 2), and this was inversely correlated with plasma FFA concentration (men, $r = -0.79$, $P = 0.019$; women, $r = -0.67$, $P = 0.017$).

Mitochondria. The most robust training-induced adaptation was an increase in the mean mitochondrial area density (men, ↑ 1.6-fold; women, ↑ 1.4-fold; $P < 0.001$; Fig. 3A) due to an increase in the size of the mitochondrial fragments (men, ↑ 2.3 fold; women, ↑ 1.7 fold; $P = 0.006$, Fig. 3C), not the number of fragments (Fig. 3B). The change in mean mitochondrial area density over the training period was significantly correlated with the change in IMCL area density ($r = 0.65$, $P = 0.043$).

Enzymes. SCHAD activity increased with training by 39% and 13% for men and women, respectively ($P = 0.003$). CS activity increased with training by 26% and 3% for men and women, respectively ($P = 0.042$). There was a significant increase in the ratio of SCHAD/CS enzyme activity with training by 25% and 12% for men and women, respectively ($P = 0.008$). There were no sex differences in the maximal enzyme activities of SCHAD or CS (Table 2).

Metabolites and hormones. There were no sex differences or training effects observed for serum insulin, triglycerides, or glucose concentration (Table 2). Resting plasma FFA ($P < 0.001$) and glycerol ($P = 0.021$) concentrations were higher for women before and following training.

DISCUSSION

The current study confirmed that healthy active women have higher IMCL compared with men (39, 42) and provided novel

Table 1. Descriptive characteristics of the subjects, dietary intake, and substrate oxidation during exercise

<table>
<thead>
<tr>
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<th>PRE</th>
<th>POST</th>
<th>PRE</th>
<th>POST</th>
<th>Training Effect</th>
<th>Sex Effect</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>24.4 (3.8)</td>
<td>22.3 (1.4)</td>
<td>23.8 (0.8)</td>
<td>22.0 (1.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.4 (3.4)</td>
<td>163.4 (4.9)</td>
<td>171.3 (3.4)</td>
<td>162.3 (4.9)</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
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<tr>
<td>BW, kg</td>
<td>79.9 (19.8)</td>
<td>64.8 (10.3)</td>
<td>64.2 (10.3)</td>
<td>64.8 (10.3)</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>FFM, kg</td>
<td>64.8 (10.3)</td>
<td>52.4 (4.6)</td>
<td>52.4 (4.6)</td>
<td>52.4 (4.6)</td>
<td>$P = 0.002$</td>
<td>$P = 0.04$</td>
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<tr>
<td>%Body fat</td>
<td>17.5 (7.7)</td>
<td>25.9 (4.2)</td>
<td>25.9 (4.2)</td>
<td>25.9 (4.2)</td>
<td>$P = 0.002$</td>
<td>$P = 0.04$</td>
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<tr>
<td>VO$_{2\text{peak}}$ l/min</td>
<td>3.33 (0.43)</td>
<td>2.39 (0.37)</td>
<td>2.39 (0.37)</td>
<td>2.39 (0.37)</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.002$</td>
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<tr>
<td>ml·kg$^{-1}$·min$^{-1}$</td>
<td>42.9 (7.3)</td>
<td>36.9 (6.6)</td>
<td>36.9 (6.6)</td>
<td>36.9 (6.6)</td>
<td>$P &lt; 0.001$</td>
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<tr>
<td>ml·kg$^{-1}$·FFM$^{-1}$·min$^{-1}$</td>
<td>51.7 (4.3)</td>
<td>49.5 (6.5)</td>
<td>49.5 (6.5)</td>
<td>49.5 (6.5)</td>
<td>$P &lt; 0.001$</td>
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Diet

Energy kcal/day | 2576 (278) | 1779 (389) | NS | $P < 0.001$ |
kJ/day          | 616 (67)   | 426 (93)   | 411 (50) | NS | $P < 0.001$ |
kcal·kg$^{-1}$·day$^{-1}$ | 33.9 (9.7) | 27.2 (4.6) | 26.9 (4.8) | NS | NS |
kcal·kg$^{-1}$·day$^{-1}$ | 8.1 (2.3)  | 6.5 (1.1)  | 6.4 (1.2)  | NS | NS |
%Fat            | 30.2 (3.7) | 25.6 (7.0) | 25.6 (7.0) | NS | NS |
%CHO            | 52.4 (6.1) | 59.3 (7.4) | 59.3 (7.4) | NS | NS |
%PRO            | 16.0 (6.1) | 14.4 (2.4) | 15.3 (3.0) | NS | NS |

Exercise

RER 0.92 (0.04) | 0.88 (0.03) | $P = 0.025$ |
%Fat oxidation  | 19 (13)    | 34 (10)    | $P = 0.018$ |
%CHO oxidation  | 77 (15)    | 64 (12)    | NS | $P = 0.032$ |

Values are expressed as means (SD). BW, body weight; FFM, fat-free mass; VO$_{2\text{peak}}$, peak oxygen consumption; CHO, carbohydrate; PRO, protein; PRE, pretraining; POST, posttraining; NS, nonsignificant. Values for exercise were the average of the last 30 minutes of cycling.
data showing that this is mainly due to a greater number of individual IMCL droplets and not larger droplets. The strong trend for an increase in IMCL area density with endurance exercise training was due to a significant increase in the number, but not size, of IMCL droplets. We have also confirmed that endurance exercise training robustly increases mitochondrial content for both men and women (15, 16), with novel data showing that it is the size, not the number, of the fragments that increases. Furthermore, we have introduced a novel concept regarding the physical relationship between mitochondria and IMCL by showing that the number of IMCL in direct contact with mitochondria increases following endurance exercise training.

A novel finding from the current study was that the sex difference in IMCL area density was mainly due to a greater number of droplets, not their individual size. Some (8, 39, 42), but not all (25, 45, 50), studies observed sex differences in IMCL content. One study found higher IMCL content in women compared with men over a wide age range (18 – 88 yr) using biochemical methods (8). A sex difference was also demonstrated with untrained, moderately trained, and endurance-trained women, all showing a higher IMCL content compared with men (42). With the use of 1H-MRS methodology, one study found that IMCL content was higher in men compared with women, and the men showed a greater decrease consequent to exercise in the rectus femoris (50); however, inspection of the individual data revealed that one male subject had an extremely high basal IMCL content that likely skewed the data. Two 1H-MRS studies found higher IMCL content in men from the soleus but not the tibialis anterior muscle (25, 45). With the use of EM analysis, one study found similar IMCL volume estimates in the vastus lateralis from five men and five women before and after 6 wk of endurance training (16). Muscle fiber type alone does not appear to influence the results, for the latter study found no statistically significant sex differences in IMCL content between the three major fiber types [I, IIA, IIB (X)] (16). Together, the above data suggest that the influence of sex on IMCL measurements is unclear but likely is a function of the specific muscle group studied (16).

The underlying mechanism(s) behind the finding of a higher IMCL in the vastus lateralis of women compared with men cannot be determined from the current data. Given that higher IMCL content has been reported in obese individuals (17, 20, 45), a higher IMCL content in women could reflect the fact that the women in the current study had higher %body fat (women = 25%; men = 17%). Although there is a positive relationship between %body fat and IMCL when comparing lean with obese individuals (17, 20, 45), this does not hold true within groups of healthy, lean individuals (45). Additionally, the IMCL content in the current study increased after training with no change in the %body fat. In obese individuals, higher IMCL is associated with a reduction in mitochondrial size, mitochondrial enzyme capacity (ETC and TCA), and β-oxidation (9, 10, 17, 20); yet mitochondrial size and enzyme capac-
ity and β-oxidation enzyme activity were not different between the men and women in the current study, and all increased after exercise training. Given that the current and many other studies (4, 14, 27, 28, 32, 43, 48) have shown that women oxidize more lipids during endurance exercise, there is evidence indicating that the higher IMCL content in women is an adaptive, regulated phenomenon, as opposed to a consequence of impaired utilization (23, 38, 40). It is possible that 17β-estradiol (E2) could explain the reported sex differences, for E2 administration in male rats increased IMCL content in red vastus muscle (6).

We have also demonstrated that the number of IMCL droplets increases following endurance exercise training, with a trend toward an increase in IMCL area density. Others have reported an increase in IMCL volume density following endurance exercise training in men and women using EM analysis in type IIA and IIB(X) fibers, with a trend in type I fibers but did not provide details on the size and number of IMCL droplets (16). A higher IMCL content has been reported in cross-sectional studies of trained vs. untrained athletes from studies using EM analysis (15) but not using direct biochemical methods (42). Endurance exercise training in lean young (41) and older (36) adults have shown increases in IMCL content. In contrast, reductions in IMCL droplet size of ~8% have been reported following exercise training in obese men and women (12). Given that the IMCL droplet size reported for obese people was 0.66–0.72 μm² (12), compared with the smaller size (<0.35 μm²) for the relatively lean and fit men and women in the current study, it is likely that exercise training reduces IMCL droplet size when they are pathologically elevated. Together, the above data are consistent with a “U”-shaped curve relating physical fitness and IMCL content (9, 24). From a metabolic perspective, it would be advantageous to have smaller IMCL droplets as opposed to the same volume occupied by fewer, larger IMCL droplets due to a higher surface/volume ratio in the smaller IMCL droplet scenario (greater surface area for proteins involved in IMCL turnover, i.e., hormone-sensitive lipase). Finally, the higher proportion of lipid oxidized during exercise in women, and the increase in lipid oxidation after training for both sexes, are consistent with the concept that a greater number of relatively small IMCL droplets are metabolically advantageous.

Our method of estimating the percent of muscle area that is occupied by IMCL or mitochondria (i.e., density) using point

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Table 2. Maximal mitochondrial enzyme activity and resting blood values

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<tr>
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<th>Men</th>
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<tr>
<td><strong>Muscle Enzyme Activity</strong></td>
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<tr>
<td>SCHAD, μmol·g wet wt^{-1}·min^{-1}</td>
<td>4.12 (1.77)</td>
<td>5.74 (1.03)</td>
<td>4.72 (1.24)</td>
<td>5.35 (0.80)</td>
<td>P = 0.003</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS, μmol·mg wet wt^{-1}·min^{-1}</td>
<td>12.93 (5.53)</td>
<td>16.34 (0.99)</td>
<td>14.88 (1.03)</td>
<td>15.30 (2.13)</td>
<td>P = 0.042</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCHAD/CS</td>
<td>0.28 (0.07)</td>
<td>0.35 (0.08)</td>
<td>0.32 (0.07)</td>
<td>0.35 (0.07)</td>
<td>P = 0.008</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resting Blood Values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>12.7 (3.3)</td>
<td>13.7 (6.5)</td>
<td>11.8 (3.8)</td>
<td>11.9 (4.1)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.7 (0.2)</td>
<td>4.5 (0.3)</td>
<td>4.5 (0.4)</td>
<td>4.6 (0.2)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>0.43 (0.25)</td>
<td>0.31 (0.10)</td>
<td>0.26 (0.05)</td>
<td>0.29 (0.08)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glyceroi, mmol/l</td>
<td>0.08 (0.07)</td>
<td>0.07 (0.02)</td>
<td>0.13 (0.02)</td>
<td>0.14 (0.08)</td>
<td>NS</td>
<td>P = 0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids, mmol/l</td>
<td>0.19 (0.10)</td>
<td>0.32 (0.11)</td>
<td>0.75 (0.29)</td>
<td>0.50 (0.18)</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). SCHAD, short-chain β-hydroxyacyl-coenzyme A dehydrogenase; CS, citrate synthase; NS, nonsignificant.
counting volume estimates (see METHODS) from a TEM 2-D image assumes that mitochondria are round or oval. This does not reflect the fact that mitochondria form mitochondrial reticulum in skeletal muscle (and are not distinct oval organelles), as described by three-dimensional EM tomography (26, 30, 31). However, our data for both mitochondria and IMCL yield nearly identical percentage values to volumes reported using grid point counting estimates (15, 16). An increase in mitochondrial area density after endurance exercise training is consistent with other studies using the EM technique (15, 16). The novel finding in the current study was that the increase in total mitochondrial area was due to an increase in the size of the mitochondrial reticulum and not the number of fragments. Howald et al. (15) found that total mitochondrial volume estimates increased by 50–60%, and just over 100% for the subsarcolemmal mitochondrial estimates, after 6 wk of endurance exercise training in men and women, and that the increases were similar for the three main fiber types (16). These observations are consistent with studies showing an increase in mitochondrial enzyme capacity using a variety of biochemical methods (5, 9, 10, 27, 33). We found an increase in SCHAD and CS activity, which is consistent with the increase in mitochondrial area, as determined using EM. A limitation of our data is the fact that we did not separate the subsarcolemmal from intramyofibrillar mitochondria, nor did we characterize fiber type; consequently, we did not analyze the mitochondria from these areas separately. The fact that our data from eight randomly chosen fibers per subject showed very similar results to those where these parameters were determined (16) suggests that our methodology yielded valid results, albeit averaged over location and between fiber types. Failure to document fiber type specifically or subcellular location does not detract from the robust finding of an approximate doubling of the mitochondrial fragment size as the main contributor to the training-induced increase in total mitochondrial area.

Another novel observation, not previously reported, was that a higher proportion of IMCL were in direct contact with mitochondria after exercise training. Although this may merely represent a higher statistical probability of the larger mitochondria coming into contact with more IMCL droplets, it could reflect an important structural-functional relationship. For example, in obese individuals or those with T2DM, it is recognized that the inability to oxidize IMCL leads to the accumulation of lipid by-products (ceramides, acyl-CoA moieties, etc.) that can inhibit insulin signaling (1, 24), and it is possible that close proximity between IMCL and mitochondria improves substrate flux from storage to metabolic sink and reduces the metabolic by-products. The concept of the proximity of hormone-sensitive lipase to the mitochondria as a determinant of FFA oxidation has been supported by in vitro studies in rat skeletal muscle (7). In support of this, in the current study, we found a higher proportion of IMCL-touching mitochondria was associated with a lower plasma FFA concentration (men, \( r = -0.79, P = 0.019 \); women, \( r = -0.67, P = 0.017 \)).

In summary, our data suggest that sex has a strong influence on IMCL content, with women having a greater number of droplets compared with men. Furthermore, endurance exercise training has a strong influence on mitochondrial area through an increase in the size of individual mitochondrial fragments that increases the number of contact sites with IMCL droplets. Endurance exercise training has a less robust influence on IMCL content, with an increase in IMCL number and a trend toward an increase in area density. Sex does not influence mitochondrial morphometry nor does it appear to influence the maximal activity of enzymes located in the mitochondrial matrix. These data are important in the design and interpretation of studies looking at IMCL and mitochondrial relationships. The potential importance between the physical relationship of IMCL droplets and mitochondrial metabolic capacity awaits further evaluation.

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REFERENCES

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