IMCL area density, but not IMCL utilization, is higher in women during moderate-intensity endurance exercise, compared with men

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Devries MC, Lowther SA, Glover AW, Hamadeh MJ, Tarnopolsky MA. IMCL area density, but not IMCL utilization, is higher in women during moderate-intensity endurance exercise, compared with men. *Am J Physiol Regul Integr Comp Physiol* 293: R2336–R2342, 2007. First published October 3, 2007; doi:10.1152/ajpregu.00510.2007.— Women use more fat during endurance exercise as evidenced by a lower respiratory exchange ratio (RER). The contribution of intramyocellular lipid (IMCL) to lipid oxidation during endurance exercise is controversial, and studies investigating sex differences in IMCL utilization have found conflicting results. We determined the effect of sex on net IMCL use during an endurance exercise bout using an ultrastructural evaluation. Men ($n = 17$) and women ($n = 19$) completed 90-min cycling at 63% $\dot{V}_{O_{2peak}}$. Biopsies were taken before and after exercise and fixed for electron microscopy to determine IMCL size, # IMCL/area, IMCL area density, and the % IMCL touching mitochondria. Women had a lower RER and carbohydrate oxidation rate and a higher lipid oxidation rate during exercise ($P < 0.05$), compared with men. Women had a higher # IMCL/area and IMCL area density ($P < 0.05$), compared with men. Women, but not men, had a higher % IMCL touching mitochondria postexercise ($P = 0.03$). Exercise decreased IMCL area density ($P = 0.01$), due to a decrease in the # IMCL/area ($P = 0.02$). There was no sex difference in IMCL size or net use. In conclusion, women have higher IMCL area density compared with men, due to an increased # IMCL and not an increased IMCL size, as well as an increased % IMCL touching mitochondria postexercise. Endurance exercise resulted in a net decrease in IMCL density due to decreased number of IMCL, not decreased IMCL size, in both sexes.

sex difference; electron microscopy; mitochondria; human

SOME (8, 14, 16, 27, 39, 44, 51, 53, 57), but not all (9, 42), studies have shown that women, compared with men, use more lipid to fuel endurance exercise, as evidenced by a lower respiratory exchange ratio (RER). More specifically, women, compared with men, have less muscle glycogen utilization (14, 51), higher glycerol turnover (8), higher plasma free fatty acid (FFA) concentration (4, 37), lower glucose rate of disappearance (Rd) (14, 16), and, although the findings are controversial, higher intramyocellular lipid (IMCL) oxidation (42, 50) during endurance exercise. It has been suggested that these sex differences during exercise are due to differences in estrogen concentration/activity (5, 31). In fact, estrogen supplementation studies in animals and humans have supported the aforementioned findings of higher fat oxidation for women during endurance exercise (7, 13, 23, 24, 30, 31, 45, 46).

Carbohydrates and fats are the main fuel sources for endurance exercise. The relative contribution, as well as the source, of carbohydrate and fat used to fuel endurance exercise, is dependent on the intensity, as well as the duration of the exercise bout (2, 43, 55). Fat oxidation contributes maximally during moderate-intensity exercise (40–65% $\dot{V}_{O_{2peak}}$) representing between 40 and 60% of total energy expenditure (55, 56). Of this, plasma FFAs contribute between 40 and 60% of total fat oxidation (43, 55, 56), and other fat sources, IMCL, and lipoprotein-derived TG, provide the rest. Under normal dietary conditions, the contribution of lipoprotein-derived TG during exercise is <10% (25), implying that IMCL supplies the remaining 30–50% of fat oxidation during moderate-intensity endurance exercise.

However, the use of IMCL during endurance exercise is controversial. Numerous studies have found that IMCL is an important substrate during moderate-intensity endurance exercise (34, 41, 47, 56, 59, 60), whereas other studies have not (3, 22, 32, 58). Additionally, several studies have found that only women use IMCL during endurance exercise (41, 42). These findings fall in line with prior research showing that women rely more on lipid sources to fuel endurance exercise compared with men, as evidenced by a lower RER (8, 14, 16, 27, 39, 44, 51, 53, 57). However, although it is plausible for women to use more IMCL during a bout of endurance exercise, it is unlikely that men do not use any IMCL as a fuel source during endurance exercise (41, 42, 50). The most likely cause of the aforementioned discrepancy are methodological limitations associated with quantifying IMCL content. There are several methods used to quantify IMCL content, including biochemical extraction, stable isotope infusion, light microscopy, electron microscopy, and ¹H-magnetic resonance spectroscopy (MRS) (54). It is generally accepted that when using the biochemical method there can be significant contamination from extramyocellular lipids (EMCL) (21). Most studies that have not shown a decrease in IMCL following a bout of endurance exercise have used the biochemical method (3, 22, 32, 58), whereas studies using stable isotope tracers (10, 35, 43, 44, 55, 56), electron microscopy (EM) (49), and ¹H-MRS (12, 34, 47, 59, 60), consistently show a decrease in IMCL following a bout of endurance exercise. Furthermore, MRS and EM appear to show similar findings when directly compared (28).

In addition to yielding IMCL content values that are biologically plausible (0.8–2.0%), the EM method also allows for the

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direct visualization of IMCL droplet size, number, and their subcellular myofibrillar location. Using the EM technique, we have shown that prior to and following a period of endurance training, women have a higher number of IMCLs in a given area of muscle, but not a greater IMCL size, compared with men, and this greater number of IMCLs contributed to the increased IMCL area density in women (52). To date, no study has looked at sex differences in IMCL use and IMCL characteristics during a bout of endurance exercise using the EM technique. The purpose of the current study was to determine the effect of sex-related metabolic differences on IMCL use during a bout of moderate-intensity endurance exercise. We also determined the effect of sex-related metabolic differences on IMCL size, IMCL number, IMCL area density, and IMCL-mitochondria proximity.

MATERIALS AND METHODS

Subjects

Men ($n = 17$) and women ($n = 19$) were recruited for the study. Men and women were matched on the basis of maximal O_2 consumption ($\dot{V}O_{2peak}$) relative to fat-free mass (ml·kg FFM^{-1}·min^{-1}) and ranged from recreationally active to endurance trained. Subject characteristics are presented in Table 1. All women were eumenorrheic, 10 were on oral contraceptives, and all were tested during the midfollicular phase of their menstrual cycle. The study was approved by the McMaster University Human Ethics Committee and was conducted in accordance with the Declaration of Helsinki. The experimental procedures, risks, and benefits were explained to each individual subject before obtaining written consent.

Study Protocol

At least 1 wk before the first trial date, subjects performed a progressive exercise test on an electronically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) to determine their $\dot{V}O_{2peak}$, as previously described (23). The $\dot{V}O_{2peak}$ was used to determine the work intensity required to elicit 65% of the subject's $\dot{V}O_{2peak}$ for subsequent testing and was confirmed by measuring $\dot{V}O_2$ at the calculated workload intensity for each subject ~30 min following the test. A relative power output of 65% $\dot{V}O_{2peak}$ was used to investigate the outcome variables during endurance exercise at moderate intensity to allow for comparisons between this and previously published papers (7, 8, 14, 36, 40, 51). Additionally, exercise performed at 65% $\dot{V}O_{2peak}$ results in a significant contribution from both carbohydrate and fat from muscle stores and plasma (43, 55).

Table 1. Subject characteristics for 17 men and 19 women who completed the study

Subject Characteristics	Men	Women
Age, yr	23 ± 1	24 ± 1
Weight, kg	75 ± 2	62 ± 2*
Body fat, %	16 ± 2	25 ± 1*
FFM, kg	63 ± 1	46 ± 1*
$\dot{V}O_{2peak}$, ml·kg^{-1}·min^{-1}	52 ± 3	44 ± 2†
$\dot{V}O_{2peak}$, ml·kg FFM^{-1}·min^{-1}	61 ± 3	58 ± 2
Dietary intake		
Total kilocalories	2458 ± 95	1888 ± 133*
% Protein	16 ± 1	14 ± 1†
% CHO	53 ± 2	59 ± 2‡
% Fat	29 ± 2	29 ± 2

Data are expressed as means $\pm$ SE. FFM, fat-free mass. *Significantly different from men, $P < 0.001$. †Significantly lower than men, $P = 0.03$. ‡Significantly higher than men, $P = 0.005$.

During the course of the study, subjects were asked to maintain and record their normal activity level. Subjects were not tested on or around major holidays or during times when their diets deviated from normal. Dietary intake was recorded and analyzed using commercially available software (Nutritionist Pro, ver. 2.2; First DataBank, San Bruno, CA).

On the morning of the test day, subjects reported to the laboratory 10–12 h postabsorptive to complete a 90-min bike ride at $63 \pm 2\%$ $\dot{V}O_{2peak}$. Body weight and height were recorded, and body composition was determined using bioelectric impedance analysis (BIA, RJL Systems BIA-101A, Mt. Clemens, MI) in half of the men and women and with dual X-ray absorptiometry (DXA, QDR 1000W, Hologic, Waltham, MA) in the remaining subjects.

Prior to and following exercise, a muscle biopsy was taken from the vastus lateralis muscle ~10 cm proximal to the knee joint using a custom suction-modified Bergström needle (5-mm diameter). Biopsies were taken from the same leg prior to and following exercise. The sample was placed in glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.4) for subsequent IMCL determination.

Respiratory measures ($\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, RER) were taken at $t = 0$, 30, 60, and 90 min during exercise using a computerized open-circuit gas collection system (Moxus Modulator $\dot{V}O_2$ system with O_2 analyzer S-3A/I and CO_2 analyzer CD-3A, AEI Technologies, Pittsburgh, PA). Whole body CHO and fat oxidation rates were estimated from expired $\dot{V}O_2$ and $\dot{V}CO_2$, as described by Peronnet and Massicotte (38): CHO oxidation = $(4.585 \times \dot{V}CO_2) - (3.226 \times \dot{V}O_2)$ and lipid oxidation = $(1.695 \times \dot{V}O_2) - (1.701 \times \dot{V}CO_2)$, where CHO oxidation and lipid oxidation are expressed in milligrams per kilogram per minute, $\dot{V}CO_2$ is the volume of CO_2 produced expressed as milliliters per kilogram per minute, $\dot{V}O_2$ is the volume of O_2 consumed expressed as milliliters per kilogram per minute. Additionally, the ratio of total CHO and lipid oxidation was also determined as follows: CHO: lipid oxidation = CHO oxidation/lipid oxidation.

IMCL Determination

Electron microscopy (EM) was used to determine IMCL characteristics as previously described (52). Briefly, fixed and embedded samples were cut into thin, longitudinal sections (70 nm) and placed on Cu/Pd grids (200 mesh size). Samples were stained for 5 min in uranyl acetate followed by 2 min in lead acetate. These analyses were performed by skilled technicians in the EM laboratory at McMaster University Medical Center. Samples were viewed at ×6500 using a transmission electron microscope (JEOL 1200 Ex, Japan), and representative pictures from each fiber (i.e., a picture that represented the IMCL and mitochondrial distributions of the whole fiber) were taken with a 1-s exposure time. One picture was taken per fiber for a total of 8–15 images per sample (1/3 images from the subsarcolemmal region near the nucleus, 1/3 from the subsarcolemmal region away from the nucleus, and 1/3 in the middle of the fiber). Pictures were digitized using a white-light illuminator (C-80 Epi-Illumination UV Darkroom, Diamed, Mississauga, ON, Canada). Mean IMCL (droplet) size, # IMCL/area, total IMCL area (%), % IMCL-touching mitochondria, the percentage of IMCL in direct physical contact with mitochondria, and net IMCL use were determined using imaging software (Image Pro Plus, Media Cybernetics, Silver Spring, MD), and the calculations are as follows: mean IMCL size (μm^2) = sum of full IMCL size/# full IMCL, # IMCL/area (#/μm^2) = total # IMCL/sum of total picture area, IMCL area density (%) = # IMCL/area × mean IMCL size × 100%, % IMCL-touching mitochondria (%) = (# IMCL touching mitochondria/total # of IMCL) × 100%, and net IMCL use (%) = [(IMCL area density pre − IMCL area density post)/IMCL area density pre × 100, where full IMCL represents any IMCL whose area is found completely within the image, and total picture area is the area of each picture that is composed of muscle. The reliability of this method has been established by our laboratory and published previously (52).

Statistical Analysis

Subject characteristics were analyzed using unpaired *t*-tests. Rest samples for RER were analyzed using unpaired *t*-tests for between-group analyses. To compare men with women, data were analyzed using two-way, mixed-design ANOVA, with sex (men vs. women) being the between-subject factor and time (pre vs. post) being the within-subject factor. A one-tailed ANOVA test was used to compare IMCL area density in men and women, because we hypothesized a priori that women would have a higher IMCL area density compared with men. When significance was attained, Tukey's honestly significant difference post hoc test was used to determine the source of the difference. Data are expressed as means $\pm$ SE. Significance was set at $\alpha = 0.05$.

RESULTS

Subject Characteristics

Subject characteristics are presented in Table 1. There was no difference in age between men and women. Women were lighter ($P < 0.001$), had a higher % body fat ($P < 0.001$), and a lower amount of fat-free mass (FFM) ($P < 0.001$). $\dot{V}_{O_{2peak}}$ ml·kg^{-1}·min^{-1} was lower in women ($P = 0.03$); however, when $\dot{V}_{O_{2peak}}$ was expressed relative to FFM, there was no difference between the sexes. Women ate fewer kilocalories ($P < 0.001$), a smaller proportion of protein ($P = 0.03$), and a greater proportion of CHO ($P = 0.005$) than men, with no difference in the proportion of kilocalories coming from fat between the sexes (Table 1).

RER

There was no difference in RER at rest between men and women. RER was lower in women during exercise, compared with men ($P = 0.03$) (Fig. 1*A*). RER was lower at 60 and 90 min of exercise compared with values at 30 min during exercise in both sexes ($P < 0.001$). CHO oxidation rate was higher in men compared with women during exercise ($P = 0.002$, Fig. 1*B*). CHO oxidation rate was lower at 60 and 90 min compared with values at 30 min during exercise in both sexes ($P < 0.001$). Lipid oxidation rate was higher in women compared with men during exercise ($P = 0.049$, Fig. 1*C*). Lipid oxidation rate was higher at 60 and 90 min compared with values at 30 min during exercise in both sexes ($P < 0.001$). Men had a higher ratio of CHO to lipid oxidation compared with women during the entire exercise bout ($P = 0.03$). When data were expressed relative to FFM, the significance was maintained.

Electron Microscopy IMCL Analyses

Mean IMCL size was similar between men and women and did not change following a 90-min bout of endurance exercise (men pre, 0.240 ± 0.028 μm^2; men post, 0.197 ± 0.014 μm^2; women pre, 0.218 ± 0.016 μm^2; women post, 0.220 ± 0.011 μm^2; Fig. 2*A*). The # IMCL/area was higher in women ($P = 0.002$) and decreased following 90 min of endurance exercise ($P = 0.03$) (men pre, 0.032 ± 0.003 #/μm^2; men post, 0.026 ± 0.002 #/μm^2; women pre, 0.042 ± 0.003 #/μm^2; women post, 0.036 ± 0.002 #/μm^2, Fig. 2*B*). IMCL area density was higher in women than in men (men pre, $0.692 \pm 0.108\%$; men post, $0.522 \pm 0.078\%$; women pre, $0.886 \pm 0.078\%$; women post, $0.747 \pm 0.071\%$, $P = 0.03$, Fig. 2*C*). IMCL area density
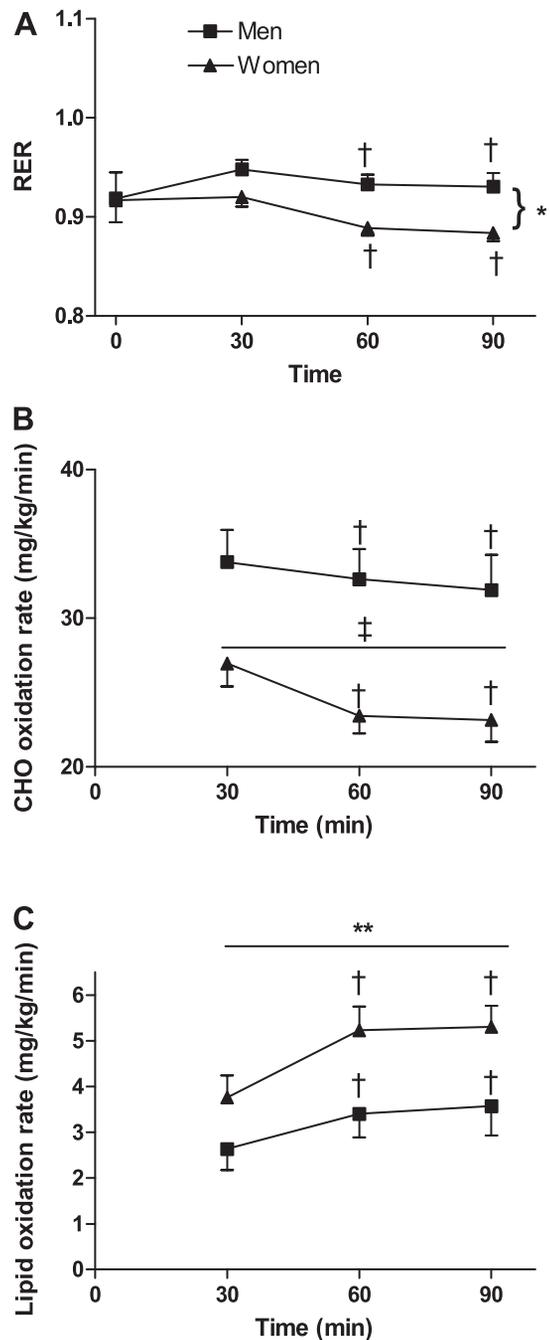
Fig. 1. Respiratory exchange ratio (RER; *A*), rate of carbohydrate oxidation (CHO; *B*), and rate of lipid oxidation (*C*) in 17 men and 19 women at rest and during a 90-min bike ride at 63% $\dot{V}_{O_{2peak}}$. Data are expressed as means $\pm$ SE. *Women lower than men, $P = 0.03$. †Different than values at 30 min; $P < 0.001$. ‡Lower than men, $P = 0.002$. **Higher than men, $P = 0.049$.

decreased following 90 min of endurance exercise in men and women ($P = 0.01$), with no difference in net IMCL utilization between the sexes. The % IMCL-touching mitochondria was not different between men and women, but it was higher in women, but not men, following the exercise bout (men pre, $81.4 \pm 2.8\%$; men post, $79.0 \pm 1.9\%$; women pre, $78.3 \pm 4.1\%$; women post, $86.2 \pm 2.6\%$, $P = 0.03$, Fig. 2*D*). Representative electron micrographs from one man and one women prior to and following the exercise bout are found in Fig. 3.
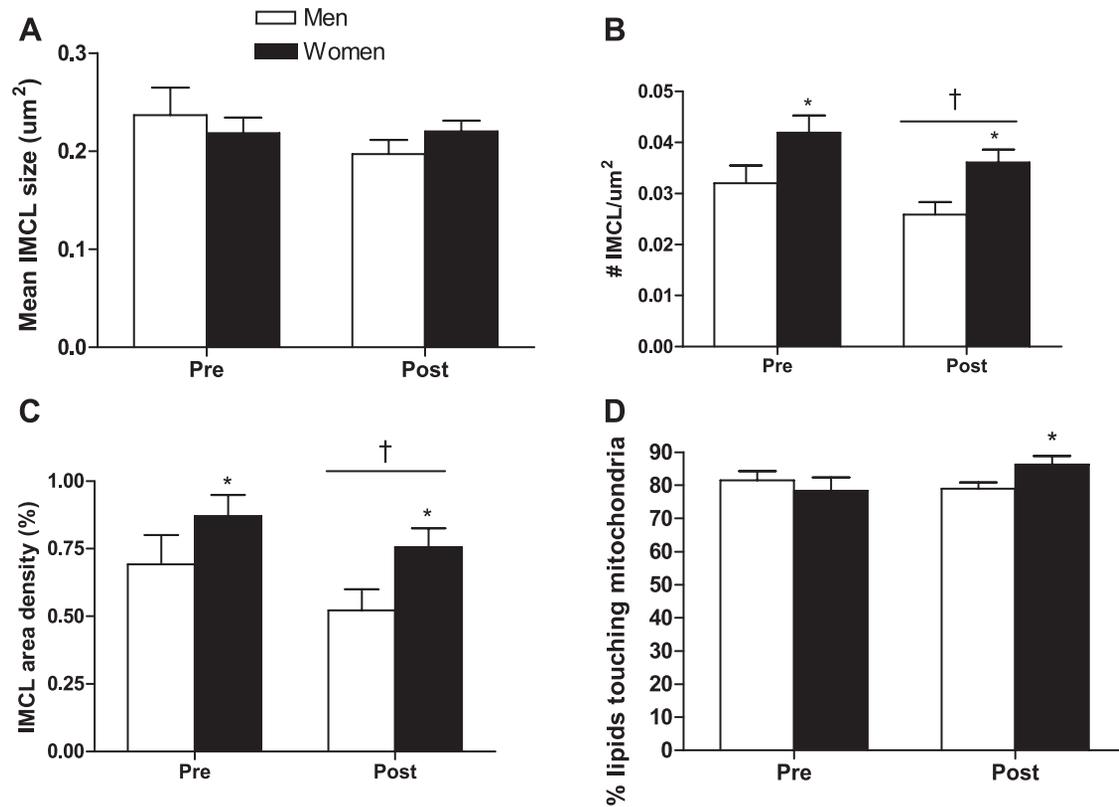
Fig. 2. Intramyocellular lipid (IMCL) characteristics prior to and following a 90-min bike ride at 63% $\dot{V}_{O_{2peak}}$. Data are expressed as means ± SE. *A*: mean IMCL size (μm^2). *B*: # IMCL/μm^2, *higher than men, $P = 0.002$. †Lower than pre-exercise, $P = 0.03$. *C*: IMCL area density (%). *Higher than men, $P = 0.03$. †Lower than pre-exercise, $P = 0.01$. *D*: % lipid-touching mitochondria. *Higher postexercise, $P = 0.03$.

DISCUSSION

The main findings of this study were that women, compared with men, had a greater IMCL area density prior to and following a bout of endurance exercise, and this was due to an increased #IMCL/μm^2 in women, not an increased IMCL size. A bout of endurance exercise decreased the #IMCL/μm^2 in women and men. Additionally, this study found that both men and women utilized IMCL during a bout of endurance exercise with no sex difference in net IMCL utilization. However, we did find that following a bout of exercise women had a greater % IMCL-touching mitochondria, whereas men did not, compared with values prior to the exercise bout.

Numerous studies have found that women have a lower RER during endurance exercise compared with men (8, 16, 27, 39, 44, 51, 53, 57). This study confirms those findings, supporting that women rely to a greater extent on lipids to fuel endurance exercise compared with their male counterparts. However, the source of the increased reliance on lipids by women during endurance exercise has yet to be elucidated.

We found no effect of sex on net IMCL utilization during a bout of endurance exercise. These findings suggest that IMCL is not the primary source of increased reliance on lipids in women during exercise in spite of three studies showing that women utilize more IMCL during a bout of endurance exercise (41, 42, 50). However, these later studies also found that men do not use any IMCL at all during the exercise bout (42, 50), a finding that was not corroborated by the current study or supported by several others using the MRS method. A limita-

tion of the studies suggesting that men do not use any IMCL during endurance exercise (41, 42, 50) is the use of the biochemical TG extraction technique to determine IMCL content and utilization during endurance exercise. As mentioned previously, the use of the biochemical method is associated with significant contamination from EMCL (21), which may lead to erroneous results. This is likely the case in at least one of the aforementioned studies, as 28% of the total lipid oxidized during the exercise bout was unaccounted for in the men (42). It is probable that the missing 28% was due to IMCL oxidation but that contamination by EMCL masked this effect. A recent sex comparative study using ^{1}H-MRS found that men relied on IMCL during exercise to a greater extent than women (60). However, a limitation of this study was that the men had a higher $\dot{V}_{O_{2peak}}$ when expressed relative to FFM, indicating that they were more trained than the women subjects, and suggesting that the increased reliance on IMCL may not be due to a sex difference but due to a difference in training status. In another sex comparative study employing ^{1}H-MRS methodology, no sex difference was found in net IMCL utilization following 60 min of cycling at 65% $\dot{V}_{O_{2peak}}$ (59); in that study, $\dot{V}_{O_{2peak}}$ was matched between men and women relative to FFM, indicating equal training status. Collectively, these findings suggest that when men and women are equally trained, there is no difference in net IMCL utilization during a bout of endurance exercise at 65% $\dot{V}_{O_{2peak}}$. However, to rule out the potential that we made a type II error, we performed a power calculation using the data from our current study and determined that we had 89% power to detect a difference in IMCL

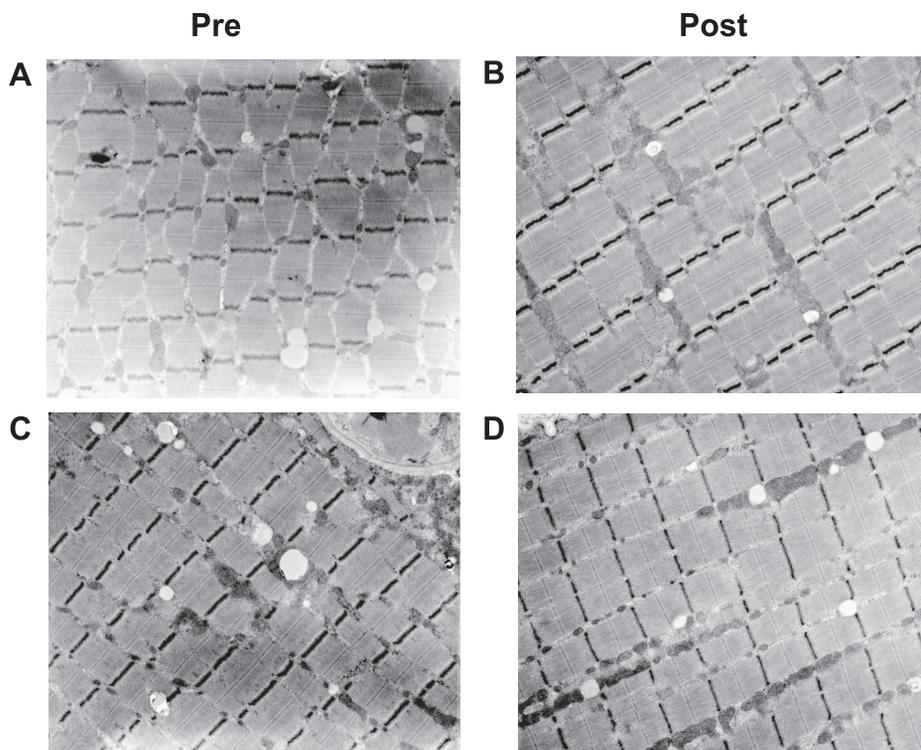**Pre**   **Post**

Fig. 3. Representative electron micrographs from one man (*A* and *B*) and one woman (*C* and *D*) prior to and following a 90-min bike ride at 63% $\dot{V}O_{2peak}$.

utilization; thus we are confident that there is no sex difference in net IMCL utilization during endurance exercise.

Several studies have found no sex difference in plasma FFA oxidation during moderate-intensity endurance exercise (6, 15, 42, 44); however, in the study conducted by Roepstorff et al. (42), women relied on 32% more plasma FFA during the last 60 min of the 90-min exercise bout compared with their male counterparts, but this difference was not statistically significant. We determined the statistical power to detect a sex difference in plasma FFA oxidation during exercise from their data (42) and found the power to be 42%; thus, the lack of difference in the aforementioned study may be the result of a type II error since a 32% greater reliance on plasma FFA is likely physiologically significant. Because a low number of subjects was used in all of the aforementioned studies (*n* = 9/group or less) it is conceivable that a type II error occurred. Indeed, in one study conducted in untrained men and women, women had a 40% greater reliance on plasma FFA oxidation during a 90-min bout of cycling performed at 50% $\dot{V}O_{2peak}$ (37). The thought that the increased reliance on lipid during endurance exercise in women is due to an increased reliance on plasma FFA stores is further supported by the finding of increased mRNA expression of FAT/CD36 and FABPpm, as well as an increased FAT/CD36 protein content in muscle from women compared with men (33), suggesting greater transport of FFA into the muscle in women. Perhaps sex differences in lipoprotein-derived TG utilization exist. To date, no study has investigated the effect of sex on lipoprotein-derived TG utilization during a bout of endurance exercise. However, a recent study showed that at rest in both the fasted and the fed state, women had a greater uptake of serum TG across the leg, compared with men (26). Whether or not this would result in greater lipoprotein-derived TG utilization during a bout of endurance exercise in women has yet to be determined. Addi-

tionally, since lipoprotein-derived TG contribute only ~10% to total fat oxidation during exercise (25), the physiological importance of this finding needs to be determined.

The unique aspect of using the EM technique when conducting IMCL analyses is that it allows for the investigation of individual IMCL characteristics. In the current study, we investigated sex differences in mean IMCL size, the number of IMCL in a given area, and the percentage of IMCL-touching mitochondria. Using EM, we were able to determine that the increased IMCL area density in women is due to an increased number of IMCL in a given area, not an increased IMCL size, supporting earlier findings from our laboratory (52). These findings support an increased "capacity" for women to utilize IMCL because an increased number of IMCL in a given area, as opposed to an increased IMCL size, allows for greater surface area and thus a greater likelihood for interaction between IMCL and mitochondria. Additionally we found that during the 90-min bout of exercise, the percentage of lipid-touching mitochondria increased in women, while there was no change in men. This finding also supports the notion that women may use or at least have an increased "capacity" to use IMCL during moderate-intensity endurance exercise. In the current study, however, there was no effect of sex on net IMCL use during a 90-min bout of moderate-intensity exercise; thus, whether this apparent increased "capacity" to use IMCL translates to an increased net IMCL use is not evident. Perhaps, this may become more apparent in ultraendurance sports, in which women are shown to have superior exercise capacity (1, 48).

Differences in estrogen concentration between men and women are the likely cause of the widely observed differences in fuel selection during endurance exercise in men and women. Estrogen supplementation studies have found that estrogen can modulate substrate utilization during endurance exercise (7, 13, 23, 46). Estrogen supplementation to amenorrheic women (46)

and men (7, 13, 23) lowered the RER (13, 23), whole body CHO oxidation (13, 23), glucose Ra, rate of disappearance (Rd), and metabolic clearance rate (7, 13, 46), and leucine oxidation (23), increased whole body fat oxidation (13, 23), and resulted in a less negative N balance following a bout of endurance exercise (23). There was no effect of estrogen supplementation on glycerol Ra or Rd (7, 46) or muscle glycogen utilization during endurance exercise (7, 13); however, estrogen supplementation did lower resting muscle glycogen concentration (13). To date, no study has investigated the effect of estrogen supplementation on IMCL utilization or IMCL characteristics. However, it appears that estrogen does influence substrate use during endurance exercise and likely mediates the sex differences observed in the current study.

Perspectives and Significance

Given the lack of consensus regarding the use of IMCL during endurance exercise, the current study provides the first direct visualization demonstrating net IMCL utilization in both men and women. Furthermore, we found that the percentage of IMCL in direct contact with mitochondria increased following exercise for women, but not men. This latter finding, in combination with our previous observation of more IMCL in contact with mitochondria after endurance exercise training, may represent structural-functional relationships that enhance IMCL substrate metabolism. The current study found no sex difference in net IMCL utilization during exercise, despite women having higher total IMCL content and total lipid oxidation during exercise. This implies sex differences must exist in intracellular FFA-IMCL flux rates or the source of lipid oxidation (plasma FFA vs. IMCL). Our findings set up future studies employing simultaneous assessment of FFA-IMCL flux rates in combination with ultrastructural assessment to determine whether the IMCL-mitochondrial relationship is an important regulator of IMCL utilization and allow us to determine the relative contribution of plasma FFA and IMCL to energy metabolism during endurance exercise in men and women. If the IMCL-mitochondrial relationship is important in regulating IMCL oxidation, it may be of clinical significance in disease states such as type 2 diabetes, in which there is dysregulated IMCL accumulation (17–20, 29).

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