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A sex-specific relationship between capillary density and anaerobic threshold

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¹Duke University Medical Center, Division of Cardiology, Durham, North Carolina; ²Department of Internal Medicine, Harbor-UCLA Medical Center, Torrance, California; ³Department of Exercise and Sports Science and Human Performance Laboratory, East Carolina University, Greenville, North Carolina; and ⁴Department of Medicine, University of Virginia, Charlottesville, Virginia

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Robbins JL, Duscha BD, Bensimhon DR, Wasserman K, Hansen JE, Houmard JA, Annex BH, Kraus WE. A sex-specific relationship between capillary density and anaerobic threshold. *J Appl Physiol* 106: 1181–1186, 2009. First published January 22, 2009; doi:10.1152/jappphysiol.90947.2008.—Although both capillary density and peak oxygen consumption ($\dot{V}O_2$) improve with exercise training, it is difficult to find a relationship between these two measures. It has been suggested that peak $\dot{V}O_2$ may be more related to central hemodynamics than to the oxidative potential of skeletal muscle, which may account for this observation. We hypothesized that change in a measure of submaximal performance, anaerobic threshold, might be related to change in skeletal muscle capillary density, a marker of oxidative potential in muscle, with training. Due to baseline differences among these variables, we also hypothesized that relationships might be sex specific. A group of 21 subjects completed an inactive control period, whereas 28 subjects (17 men and 11 women) participated in a 6-mo high-intensity exercise program. All subjects were sedentary, overweight, and dyslipidemic. Potential relationships were assessed between change in capillary density with both change in $\dot{V}O_2$ at peak and at anaerobic threshold with exercise training. All variables and relationships were assessed for sex-specific effects. Change in peak $\dot{V}O_2$ was not related to change in capillary density after exercise training in either sex. Men had a positive correlation between change in $\dot{V}O_2$ at anaerobic threshold and change in capillary density with exercise training ($r = 0.635$; $P < 0.01$), whereas women had an inverse relationship ($r = -0.636$; $P < 0.05$) between the change in these variables. These findings suggest that, although enhanced capillary density is associated with training-induced improvements in submaximal performance in men, this relationship is different in women.

skeletal muscle; exercise; studies of a targeted risk reduction intervention through defined exercise; peak oxygen consumption

IT IS WIDELY ACCEPTED THAT the oxidative capacity of skeletal muscle can be increased with aerobic exercise training. Reported changes include increases in oxidative enzyme activity, mitochondrial volume and number, and transitions in muscle fiber type (1, 8, 12, 15, 18). Capillary supply is different between trained and untrained subjects (19) and has the adaptive ability to increase after moderate to vigorous aerobic training (2, 20, 24). A larger capillary network provides for a longer mean transit time and greater surface

area for optimal exchange of gases, substrates, and metabolites (32).

Although many studies show an increase in both skeletal muscle capillary supply and peak oxygen consumption ($\dot{V}O_2$) after exercise training, very few of these studies have suggested a significant relationship between capillarity and peak $\dot{V}O_2$ (17, 31). This may be explained by the body of work that suggests the central pump and lungs, rather than skeletal muscle, limit maximal $\dot{V}O_2$ (14, 30, 33). Thus, although central hemodynamics are more responsible for determining maximal measurements (peak $\dot{V}O_2$), it has been suggested that peripheral metabolic indexes may relate more to submaximal measures (anaerobic threshold) (3, 4, 13, 21).

Numerous studies have reported on the physiological differences among genders. However, there is sparse literature comparing skeletal muscle characteristics of men and women, and fewer studies examining the potential for differential exercise training effects on skeletal muscle among men and women. Therefore, the purpose of this study was twofold: 1) to determine whether change in skeletal muscle capillary density is more related to change in $\dot{V}O_2$ at anaerobic threshold than peak $\dot{V}O_2$ with exercise training and 2) to explore sex-specific changes in capillary density and its relationship to improvements in measures of maximal and submaximal exercise capacity.

METHODS

Subject population. The inactive control group consisted of 21 subjects (9 men and 12 women), and the exercise training group included 28 subjects (17 men and 11 women). These subjects were a cohort from the studies of a targeted risk reduction intervention through defined exercise (STRIDE) trial (26). Subjects were randomly assigned into groups. All subjects were overweight (body mass index of 25–35 kg/m²), sedentary adults between 40 and 65 yr of age with no known history of or clinical evidence for cardiovascular disease, diabetes, or hypertension. All women were postmenopausal. Inclusion criteria required an elevated LDL-cholesterol concentration (≥ 130 and ≤ 190 mg/dl) or a decreased HDL-cholesterol concentration (< 40 mg/dl for men and < 45 mg/dl for women). Subjects were nonsmokers and were not taking any lipid-lowering medications. Subjects were asked not to alter their diet and were excluded if they gained or lost $\geq 2.5\%$ of their body weight during the study period. The research protocol was approved by all relevant institutional review boards, and each subject provided written, informed consent before participation.

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Exercise training. The exercise group was assigned to a program calorically equivalent to ~20 miles (32.0 km) of jogging per week at 65–80% of individual peak $\dot{V}O_2$. The specific exercise prescription was to expend 23 kcal·kg body wt⁻¹·wk⁻¹ (26). There was an initial ramp period of 2–3 mo in which exercise duration and exercise intensity were gradually increased until the appropriate exercise prescription was obtained. The initial ramp was followed by an additional 6 mo of training at the appropriate exercise prescription. The control group did not participate in an exercise program but completed all testing again at the end of a 6-mo inactive period.

Exercise testing. All subjects underwent a maximal cardiopulmonary exercise test (CPET) with a 12-lead electrocardiogram and expired gas analysis on a treadmill. These tests were performed twice at baseline and once postintervention. The protocol consisted of 2-min stages, increasing in workload by ~1 metabolic equivalent per stage. Expired gases were analyzed continuously using a Sensesmedics 2900 unit (Yorba Linda, CA) or Parvo Medics unit (Sandy, UT). The same metabolic cart was used for all tests for each subject. The last 40 s were averaged to determine peak $\dot{V}O_2$.

Anaerobic threshold readings. Anaerobic threshold was determined using the V-slope method (5). Twenty-second averages of $\dot{V}O_2$ and $\dot{V}CO_2$ were obtained via the mixing chamber from the metabolic cart. Three separate experienced readers were given, unidentified and in random order, the 20-s averaged plots of $\dot{V}O_2$ vs. carbon dioxide production for each completed CPET (pre- and postintervention) and asked to mark the point of anaerobic threshold. The $\dot{V}O_2$ (l/min) at the point of anaerobic threshold was identified and recorded. For a value to be considered valid, two of three readers had to agree within a variance of 5%. If two or all three readers were in agreement, the values were averaged. If no two readers were within 5%, the $\dot{V}O_2$ at anaerobic threshold was considered indeterminate, and the subject's data were not included in the analysis.

Muscle biopsies. Biopsy samples were obtained from the vastus lateralis muscle using a modified Bergstrom needle technique (Bergstrom). Biopsy sites were anesthetized with a 2% lidocaine solution, and 0.5-cm incisions were made through the skin and fascia lata. The needle was consistently inserted to a depth of 40–60 mm. Samples were then mounted in cross section in optimal cutting temperature (OCT) compound (Miles Pharmaceutical, West Haven, CT) beds and snap frozen at –80°C.

Histological analysis. The capillary density of each section was determined in two ways: 1) the ratio of endothelial cells to muscle fibers, calculated by dividing the total number of CD31-positive cells by the total number of muscle fibers; and 2) the number of endothelial cells per square millimeter, calculated by dividing the total number of CD31-positive cells by the area (mm²) of tissue, which was measured with a standard hemocytometer and NIH Image J software. A minimum of 100 muscle fibers were counted per sample for both endothelial cell-to-fiber ratio and capillary density per square millimeter. Endothelial cells were identified in histological sections using immunohistological techniques with an established endothelial cell-specific monoclonal antibody in methods previously described (10).

Statistical analysis. Subjects without pre and post values for peak $\dot{V}O_2$ ($n = 0$), anaerobic threshold ($n = 6$), and capillary density ($n = 5$) were not included in the analysis. Data were considered unusable if exercise training compliance was below 74% ($n = 4$), peak RER for any CPET was below 1.05 ($n = 2$), or the CPET was terminated due to reasons other than volitional fatigue (i.e., orthopedic limitation). The remaining subjects were included in the study analysis. Due to baseline differences in peak $\dot{V}O_2$, capillary density, and anaerobic threshold between men and women, separate analyses were performed based on sex.

Independent *t*-tests were used to determine any demographic and physiological differences between groups (control vs. exercise) and between the sexes at baseline. Baseline to postintervention differences for each variable were determined using paired *t*-tests for control women, control men, exercise women, and exercise men. ANOVA testing was used to test for baseline to postintervention differences (relative percent change scores) between groups. In addition, a two-way ANOVA using both sex and training group as factors was conducted to evaluate whether men and women responded differently to training. Pearson correlations were used to analyze the relationships between the changes in capillary density and both anaerobic threshold and peak $\dot{V}O_2$. All tabular means are presented as mean \pm SD. A *P* value of <0.05 was considered significant for all tests.

RESULTS

Baseline. There were no significant differences between the control and exercise groups at baseline. However, men had a greater baseline peak $\dot{V}O_2$ and a higher $\dot{V}O_2$ at anaerobic threshold than did women. Men also had a greater capillary-to-fiber ratio than women. However, capillary density per square millimeter was not significantly greater in men. Baseline characteristics are shown in Table 1.

Effects of exercise training. The effects of exercise training are presented in Table 2. Men and women in the control group had no changes in peak $\dot{V}O_2$, capillary density, or anaerobic threshold over the testing period. Exercise-trained men and women significantly increased their peak $\dot{V}O_2$ with training, and men improved more than women ($P < 0.05$). Exercise-trained women had a significant increase in capillary density (capillary-to-fiber ratio as well as capillary density per square millimeter) with training, whereas the men did not. Although the men trended toward having a greater improvement (not significant difference), both men and women had a significant absolute increase in $\dot{V}O_2$ at anaerobic threshold. However, anaerobic threshold expressed as a percentage of peak $\dot{V}O_2$ was unchanged from baseline (58.3 ± 5.3 and $69.0 \pm 8.4\%$) to posttraining (58.6 ± 5.8 and $68.0 \pm 6.1\%$) for both men and women, respectively. There were significant interaction effects (sex \times training group) for change in peak $\dot{V}O_2$, $\dot{V}O_2$ at anaer-

Table 1. Baseline characteristics divided by both group and sex

	Control ($n = 21$)	Exercise ($n = 28$)	Men ($n = 26$)	Women ($n = 23$)
Age, yr	52.2 \pm 6.6	52.1 \pm 6.6	51.0 \pm 7.8	53.5 \pm 4.6
Race, % Caucasian	76%	89%	92%	74%
BMI, kg/m ²	29.9 \pm 3.9	30.1 \pm 3.2	30.5 \pm 3.5	29.4 \pm 3.4
Peak $\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	27.2 \pm 5.6	28.4 \pm 5.4	31.4 \pm 4.4	23.9 \pm 3.4*
Peak $\dot{V}O_2$, l/min	2.3 \pm 0.7	2.6 \pm 0.7	3.0 \pm 0.5	1.8 \pm 0.2*
$\dot{V}O_2$ at AT, l/min	1.5 \pm 0.4	1.6 \pm 0.3	1.8 \pm 0.3	1.2 \pm 0.1*
Capillary density, endothelial cell-to-fiber ratio	1.5 \pm 0.3	1.6 \pm 0.4	1.6 \pm 0.4	1.4 \pm 0.3*
Capillary density, endothelial cells/mm ²	307.4 \pm 63.5	348.3 \pm 92.5	347.9 \pm 95.5	309.8 \pm 60.9

Values are means \pm SD. $\dot{V}O_2$, oxygen consumption; BMI, body mass index; AT, anaerobic threshold. *Men were significantly different ($P < 0.05$) from women.

Table 2. Effects of exercise training/control period in men and women

	Control Men (n = 9)			Exercise-Trained Men (n = 17)			Control Women (n = 12)			Exercise-Trained Women (n = 11)		
	Baseline	Posttraining	Mean % change	Baseline	Posttraining	Mean % change	Baseline	Posttraining	Mean % change	Baseline	Posttraining	Mean % change
Peak $\dot{V}O_2$, ml·kg ⁻¹ ·min ⁻¹	32.1±3.9	31.1±4.3	-2.9±3.3	31.0±4.8	37.5±5.7*†	21.2±10.1*	23.5±3.4	23.6±3.0	0.6±6.0	24.3±3.5	24.7±3.6*†	13.1±4.7*
Peak $\dot{V}O_2$, l/min	3.00±0.5	2.95±0.5	-1.9±3.8	3.01±0.5	3.54±0.5*†	18.0±8.5*	1.82±0.2	1.85±0.2	1.5±4.7	1.86±0.2	2.06±0.2*†	10.9±5.5*
$\dot{V}O_2$ at AT, l/min	1.88±0.4	1.84±0.4	-1.6±7.1	1.75±0.2	2.08±0.4*†	18.8±11.6*	1.18±0.1	1.25±0.2	5.9±11.2	1.27±0.1	1.40±0.2*†	10.2±14.5*
Capillary density, endothelial cell-to-fiber ratio	1.49±0.4	1.52±0.4	4.1±11.4	1.70±0.5	1.79±0.3	7.7±15.4	1.42±0.3	1.46±0.3	2.5±9.6	1.43±0.3	1.84±0.4*†	31.2±23.3*
Capillary density, endothelial cells/mm ²	315.0±8	322.4±60	4.1±11.5	366.3±9	386.7±70	8.3±15.7	301.7±5	311.0±65	3.0±9.6	319.6±7	397.5±76*†	27.3±26.0*

Values are means ± SD. The % change columns reflect the mean of the individual percent change for each subject. *Exercise-trained men were significantly different from control men, and exercise-trained women were different from control women ($P < 0.05$). †Baseline values were significantly different ($P < 0.05$) from postexercise values.

obic threshold, and capillary density (both capillary-to-fiber ratio and endothelial cells per square millimeter) ($P < 0.05$). There were no differences in exercise adherence (89 ± 7.2 vs. $91 \pm 6.8\%$), frequency (3.8 ± 0.5 vs. 4.0 ± 0.5 average days/wk), training intensity (74 ± 4.9 vs. $72 \pm 5.2\%$ of peak $\dot{V}O_2$), or time (210 ± 38.0 vs. 218 ± 28.2 min/wk) between men and women, respectively.

Relationship between capillary density and peak $\dot{V}O_2$ and anaerobic threshold. No relationships were observed between peak $\dot{V}O_2$ and capillary density at baseline or between change in peak $\dot{V}O_2$ and capillary density in response to training for men or women. Men displayed a significant positive relationship between percent change in $\dot{V}O_2$ at anaerobic threshold and percent change in capillary density with exercise training ($r = 0.635$, $P < 0.01$ for capillary-to-fiber ratio; and $r = 0.62$, $P < 0.01$ for endothelial cells per square millimeter), whereas women displayed a significant inverse relationship ($r = -0.636$, $P < 0.05$ for capillary-to-fiber ratio; and $r = -0.64$, $P < 0.05$ for endothelial cells per square millimeter). Although the relationship between the exercise-induced change in capillary density and $\dot{V}O_2$ at anaerobic threshold was negative for women, the changes were both positive. These relationships are illustrated in Fig. 1.

DISCUSSION

There has been a long-standing debate concerning whether central hemodynamics or oxidative properties of skeletal muscle play a major role in limiting peak $\dot{V}O_2$ (30, 39). Our findings confirm that, over a period of exercise training, capillary density is not related to peak $\dot{V}O_2$ but rather is related to change in a measure of submaximal performance, anaerobic threshold. Examination of gender-specific relationships between capillarity and anaerobic threshold with training indicates that women may respond differently than men. This finding suggests that, although capillary density may be an integral player in the response of anaerobic threshold in men, something other than capillary density likely contributes to training-induced improvements in anaerobic threshold in women.

To the best of our knowledge, no one has reported on the relationship between capillary density and anaerobic threshold with or without training. However, limited literature does link anaerobic threshold to other measures of skeletal muscle oxidative capacity. Several investigators have observed that skeletal muscle oxidative enzymes correlate with anaerobic threshold (3, 16, 29). The percentage of slow-twitch muscle fibers, which are high in mitochondrial enzyme activity, has also been positively related to anaerobic threshold (4, 21). Such data suggest that the accumulation of lactate may be dependent on the oxidative machinery of skeletal muscle. This research also implies that muscle metabolic profile is more closely connected to measurements of submaximal endurance than to maximal aerobic power. Although many have come to this conclusion (3, 4, 14), these findings have been based on research involving primarily or exclusively men. Consequently, less is known about these relationships in women and how they may respond to exercise training. Although our data examining muscle capillarity endorse the previous theory that metabolic markers of skeletal muscle are related to anaerobic threshold in men, we

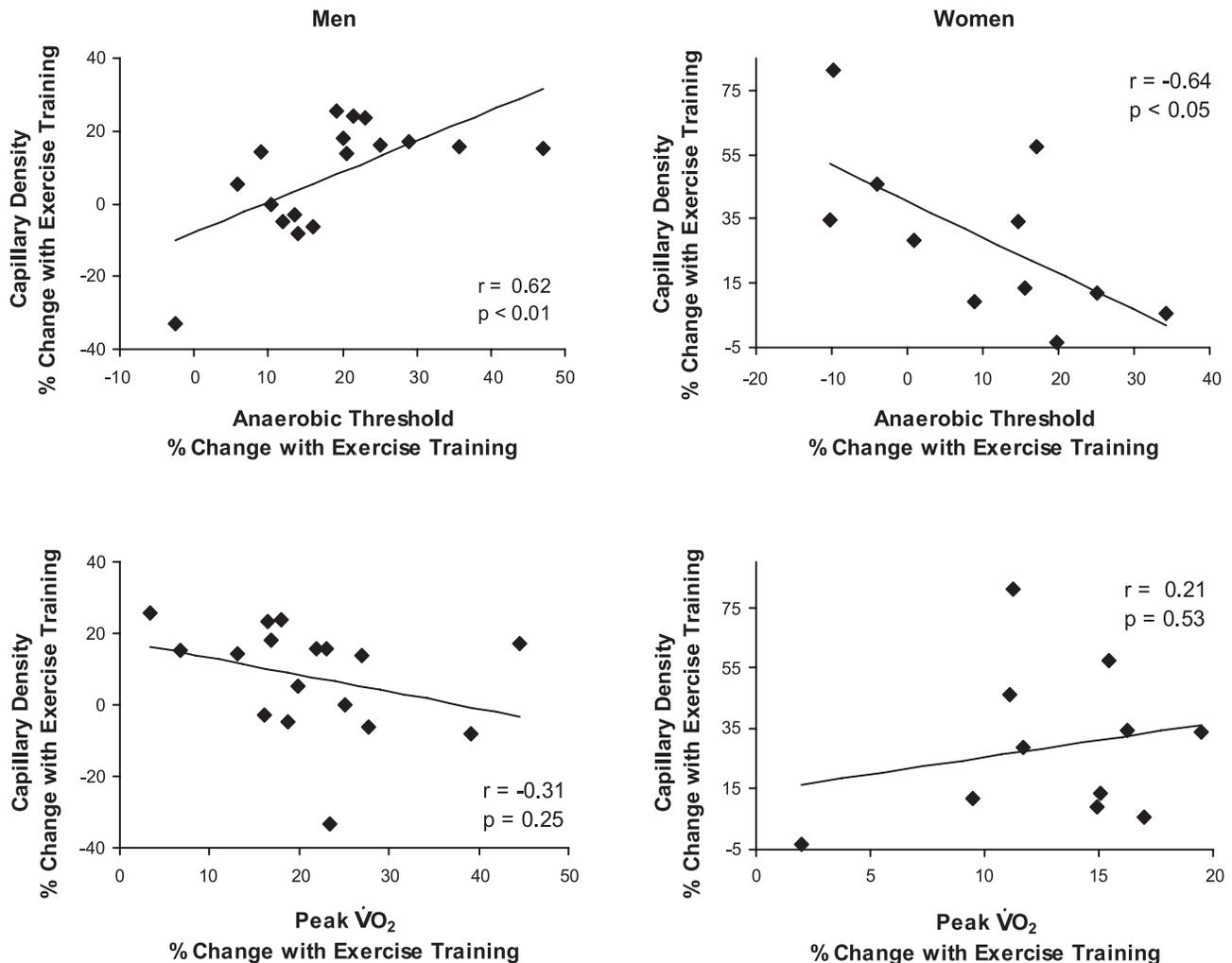


Fig. 1. The relationship of the percent change in capillary density (endothelial cells/mm²) and the percent change in functional measures with exercise training. Men had a significant positive correlation between the percent change in oxygen consumption ($\dot{V}O_2$) at anaerobic threshold and the percent change in capillary density with exercise training ($r = 0.62$; $P < 0.01$), whereas women had a significant inverse relationship ($r = -0.64$; $P < 0.05$). The relationship of the percent change in capillary density (endothelial cells/mm²) and the percent change in peak $\dot{V}O_2$ with exercise training was not significant for men or women.

add the novel finding that this relationship is opposite in women (Fig. 1).

Men and women tend to increase peak $\dot{V}O_2$ in a similar fashion in response to exercise training (11, 25). It has been suggested, however, that the mechanism responsible for the increase in peak $\dot{V}O_2$ may differ between men and women. Spina et al. (34) reported that, in men, two-thirds of the increase in peak $\dot{V}O_2$ after exercise training appears to be accounted for by improved stroke volume and one-third via arteriovenous O_2 differences. In contrast, women rely on increases in differences to improve peak $\dot{V}O_2$ (34). Coggan et al. (8) have also reported that the improved aerobic capacity in older women with exercise training is mediated entirely by skeletal muscle adaptations. Although in our study we cannot report on the central cardiovascular contribution, if any, to the improvements observed in peak $\dot{V}O_2$, our data do not demonstrate a positive relationship between skeletal muscle capillary density and peak or submaximal functional capacity in women, as may have been expected from these previously quoted studies. It is possible that alternate peripheral measures of oxygen extraction and utilization are responsible for these prior findings.

As previously reported (7, 8), we observed baseline capillary density to be lower in women than in men (Table 1). Although this might predict that the skeletal muscle of untrained women is less oxidative than men, fiber typing and substrate utilization research has consistently proven otherwise. Consistent with this research, we observed that women reach anaerobic threshold at a higher percentage of peak $\dot{V}O_2$ than did men (58.6 vs. 68.0%; $P < 0.05$). As noted, the adaptive response of capillary density to exercise training appears to be different between men and women. Even though men increased their peak $\dot{V}O_2$ and tended to increase their anaerobic threshold more than women in this study, women increased their capillary density fourfold compared with men with training (31.2 vs. 7.7%). In a slightly older population, using a similar exercise stimulus, Coggan et al. (8) also showed that women increased their capillary density (expressed per fiber) with training more than men, but the difference between the sexes was not as great (38 vs. 21%). The less robust response in men compared with that of Coggan et al. and some others may be due to differences in staining methodology, the muscle bed sampled relative to the exercise stimulus, or the mode of exercise training (2, 24). Taken together, these findings

support that the capillarity of skeletal muscle in women is more sensitive to change with exercise training than is that of men. However, the functional significance of these changes remains unclear.

Despite a profound increase in capillarity with exercise training, women, unlike men, do not appear to depend on this adaptation for the improvement in anaerobic threshold. Quite interestingly, the relationship between the changes in these two variables is negative in women. That is, those with the largest increase in capillarity with exercise training tend to have the smallest increase in anaerobic threshold. Although the mechanism for this response is unknown, there are many potential areas worthy of investigation. It has been consistently demonstrated that women oxidize proportionately more lipid and less carbohydrate during endurance exercise than men (6, 36, 37). Although controversial, several studies have observed that women have an enhanced use of intramyocellular lipid during endurance exercise (27, 28, 35). Although studies in men have shown that a dense capillary network is critical for the uptake of lipids by the skeletal muscle (23, 32), it is possible that, with training, women rely more on an enhanced supply of intramuscular lipid and less on their capillary network to oxidize fat during submaximal exercise. This might provide a potential explanation for our finding that larger improvements in anaerobic threshold correspond with smaller increases in capillary density in women with endurance training.

Our findings contribute to the current literature regarding the impact of skeletal muscle capillarity on peak and submaximal exercise performance. These data support the theory that skeletal muscle capillary density is not integral to changes in peak $\dot{V}O_2$ with training. However, skeletal muscle capillary density is significantly related to changes in anaerobic threshold with endurance training. We also observed that, in women, a previously understudied population, this relationship is opposite from that of men. This finding is preliminary and requires further study and confirmation in a larger group of women. Although the precise mechanism remains unclear, the sex-specific relationship between capillary density and anaerobic threshold could have important influences on innovative approaches to exercise training. Future studies should be powered to further explore these differences.

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