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## Lung density is not altered following intense normobaric hypoxic interval training in competitive female cyclists

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**Guenette JA, Sporer BC, MacNutt MJ, Coxson HO, Sheel AW, Mayo JR, McKenzie DC.** Lung density is not altered following intense normobaric hypoxic interval training in competitive female cyclists. *J Appl Physiol* 103: 875–882, 2007. First published June 14, 2007; doi:10.1152/jappphysiol.00247.2007.—Noninvasive imaging techniques have been used to assess pulmonary edema following exercise but results remain equivocal. Most studies examining this phenomenon have used male subjects while the female response has received little attention. Some suggest that women, by virtue of their smaller lungs, airways, and diffusion surface areas may be more susceptible to pulmonary limitations during exercise. Accordingly, the purpose of this study was to determine if intense normobaric hypoxic exercise could induce pulmonary edema in women. Baseline lung density was obtained in eight highly trained female cyclists (mean  $\pm$  SD: age =  $26 \pm 7$  yr; height =  $172.2 \pm 6.7$  cm; mass =  $64.1 \pm 6.7$  kg;  $\dot{V}O_{2\max} = 52.2 \pm 2.2$  ml $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ ) using computed tomography (CT). CT scans were obtained at the level of the aortic arch, the tracheal carina, and the superior end plate of the tenth thoracic vertebra. While breathing 15% O<sub>2</sub>, subjects then performed five 2.5-km cycling intervals [mean power =  $212 \pm 31$  W; heart rate (HR) =  $94.5 \pm 2.2\%$ HR<sub>max</sub>] separated by 5 min of recovery. Throughout the intervals, subjects desaturated to  $82 \pm 4\%$ , which was 13  $\pm$  2% below resting hypoxic levels. Scans were repeated 44  $\pm$  8 min following exercise. Mean lung density did not change from pre ( $0.138 \pm 0.014$  g/ml)- to postexercise ( $0.137 \pm 0.011$  g/ml). These findings suggest that pulmonary edema does not occur in highly trained females following intense normobaric hypoxic exercise.

lung water; hypoxia; computed tomography

THE MAMMALIAN PULMONARY blood-gas barrier permits efficient gas exchange via passive diffusion because of its extremely thin membrane and vast surface area. The extreme thinness makes the blood-gas barrier particularly vulnerable to ultrastructural damage (43). Stress failure leading to pulmonary edema may occur because of the high capillary wall stresses encountered during intense exercise. Indeed, it has been known for centuries that Thoroughbred racehorses have blood in their nostrils following exercise (45). In fact, it has been shown that Thoroughbreds in training contain hemosiderin-laden macrophages in their tracheal washings (48), indicating that these animals are developing pulmonary edema due to strenuous exercise. This is likely attributable to their extraordinarily high cardiac outputs, which can result in pulmonary capillary pressures of 100 mmHg (43). Elite human athletes are not capable

of achieving such high pressures, but it has been suggested that their capillary pressures can exceed 35 mmHg near the base of the lungs (47). It has been shown experimentally that when subjected to similar pressures, the blood-gas barrier in the rabbit can undergo ultrastructural changes (41). There is some evidence to suggest that the integrity of the blood-gas barrier is altered in healthy human athletes during exercise. For example, Hopkins et al. (20, 21) found an increase in red blood cells and protein in bronchoalveolar lavage fluid following 7 min of maximal intensity cycle exercise but not during prolonged submaximal exercise.

Younes et al. (49) demonstrated that it takes approximately a fourfold increase in cardiac output to increase lung water in isolated in situ perfused left upper lobes in dogs. It is well known that trained human athletes can increase their cardiac output five to eight times resting levels during exercise (10). This fact, coupled with the work of Younes et al. (49) suggests that trained athletes may be more likely to develop pulmonary edema compared with their untrained counterparts. However, the development of pulmonary edema during exercise in athletes is not a universal finding. For example, Hanel et al. (14) showed that decrements in the pulmonary diffusion capacity for carbon monoxide (DL<sub>CO</sub>) and the membrane diffusion capacity following 6-min bouts of all-out rowing was influenced by pulmonary capillary blood volume rather than pulmonary edema.

Diagnostic imaging techniques have recently been used as an alternative and noninvasive method of examining pulmonary edema following exercise. Previous studies using chest x-ray have shown that pulmonary edema occurs following exercise at altitude ( $\sim$ 2,400 m; Ref. 2) and during ultramarathon running (29) but not during progressive cycle exercise to exhaustion (11). Recent advances in diagnostic imaging technology such as computerized tomography (CT) and magnetic resonance imaging (MRI) permit an examination of pulmonary edema by measuring changes in lung density following exercise. However, there remains considerable uncertainty as to whether or not pulmonary edema occurs during exercise despite the use of these sensitive imaging techniques. Several studies using CT show no increase in lung density following exercise (26, 27, 40) while others using CT (3) and MRI (30) do show a change. This discrepancy may be attributable to differences in exercise duration and/or intensity,

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different imaging techniques, subject fitness levels, or the timing of the postexercise image scans.

We recently conducted a study to determine if lung density could be increased following intense hypoxic interval training in male endurance athletes using CT (26). The protocol was designed to maximize both intensity and duration and was combined with a hypoxic stimulus [ $FI_{O_2} = 15\%$  ( $\sim 2,700$  m)], which is known to increase pulmonary artery pressure via heterogeneous hypoxic vasoconstriction (12, 44). Despite the extreme physiological stress, we were unable to detect a change in lung density following exercise. In contrast, a recent study by Zavorsky et al. (51) found evidence of pulmonary edema following intense normoxic interval training in aerobically trained women using chest radiography. This study raises important questions related to potential sex differences in the pulmonary response to exercise. There have been a number of studies that suggest women may be more susceptible to pulmonary limitations during exercise such as exercise-induced arterial hypoxemia relative to men (15, 33). Women typically have smaller lung volumes, smaller diameter airways, and a smaller lung diffusion surface area relative to men of equal stature (19, 37), which may predispose them to gas exchange abnormalities during exercise. Zavorsky et al. (51) found that 9 of 14 women developed pulmonary edema following high-intensity sea level interval training. We asked if the discrepancy between this study and our previous work in men (26) was attributable to a sex difference. Therefore, we determined if highly trained women could develop pulmonary edema as determined via CT following normobaric hypoxic exercise. We hypothesized that there would be a significant increase in lung density following intense hypoxic interval training in competitive female cyclists.

## METHODS

**Subjects.** All experimental procedures were approved by the Clinical Screening Committee for Research at the University of British Columbia and conformed to the Declaration of Helsinki. All subjects gave written, informed consent prior to participating in the study.

Eight competitive female endurance cyclists were recruited from the university campus and through local cycling organizations. All subjects were nonasthmatic, nonsmokers, and had no history of cardiopulmonary disease. Subjects trained regularly (4–7 days/wk), were experienced at interval training, competed in road or mountain cycling at the provincial, national, or international level and had a maximal aerobic capacity  $>120\%$  of predicted values. All subjects were tested randomly throughout the menstrual cycle and the use of oral contraceptives did not result in exclusion from this investigation.

**General protocol.** The experimental protocol included two testing days separated by a minimum of 72 h. Subjects were asked to refrain from vigorous exercise for 24 h and caffeine for 12 h prior to each testing session. On *day 1*, subjects reported to the laboratory and underwent basic anthropometric measures followed by spirometry testing and an incremental cycle test to exhaustion to determine maximal aerobic capacity ( $\dot{V}O_{2max}$ ). On *day 2*, subjects underwent a baseline limited low dose CT scan of their lungs. They then performed a hypoxic interval training session on a cycle ergometer followed by another CT scan using the same slice location and acquisition parameters.

**Spirometry and  $\dot{V}O_{2max}$  (*day 1*).** Basic pulmonary function was determined using an automated ventilatory analysis system (Medical Graphics Metabolic Cart, CPX-D). Measurements included forced vital capacity (FVC), forced expired volume in 1 s ( $FEV_{1.0}$ ), and  $FEV_{1.0}/FVC$ . Measurements were obtained with subjects seated using

standard protocols, and results are expressed using prediction equations (1). Subjects with a  $FEV_{1.0}/FVC < 80\%$  of predicted were excluded from this investigation. Subjects then performed a 5-min self-selected warm-up on a Velotron Pro cycle ergometer (Racermate, Seattle, WA) followed by a progressive exercise test starting at 0 W with the workload increasing in a ramp fashion by 30 W every minute until volitional fatigue. The test was terminated when the subject could no longer maintain a cadence of 60 rpm. During exercise, subjects wore a nose clip and breathed through a mouthpiece connected to a low-resistance non-rebreathing valve (model 2700B, Hans-Rudolph, Kansas City, MO). Mixed expired gas concentrations and ventilatory parameters were measured continuously using an automated gas analysis system (TrueOne 2400, Parvo Medics, Provo, UT) and values were recorded over 15-s epochs. The gas analyzers were calibrated prior to each test using known gas concentrations, and the pneumotachometer was calibrated using a 3-liter calibration syringe over a range of flow rates to ensure linearity. Oxyhemoglobin saturation ( $Sa_{O_2}$ ) was measured using a finger or ear pulse oximeter (Nonin 8600, Medical, Minneapolis, MN) and recorded every 30 s. Heart rate was continuously assessed by telemetry (Polar Vantage XL, Kempele, Finland) and recorded every 15 s.  $\dot{V}O_{2max}$  was determined by averaging the two highest consecutive 15-s values.

**Hypoxic intervals (*day 2*).** Subjects performed a 20-min self-selected warm-up in normoxia on the Velotron cycle ergometer. This warm-up protocol was used because it is similar to what these athletes would perform prior to an intense interval training session. The ergometer was adjusted to the riders' exact size specifications prior to testing. Subjects then breathed through a face mask connected to a low-resistance non-rebreathing valve (model 2700B, Hans-Rudolph). The inspiratory tube was connected to a reservoir of humidified hypoxic gas (15%  $O_2$ ; balance  $N_2$ ) and the expiratory tube was connected to a heated pneumotachometer (model 3813, Hans Rudolph), a mixing chamber, and the gas analysis system (TrueOne 2400, Parvo Medics). Metabolic parameters, heart rate, and  $Sa_{O_2}$  were monitored in the same manner as *day 1*. Following the 20-min warm-up, subjects sat quietly on the ergometer for 5 min so that hypoxic resting data could be obtained. They then performed five 2.5-km intervals at maximal effort separated by 5 min of active recovery. Previous pilot work showed that near maximal intensity (i.e.,  $\sim 95\%$  of maximum heart rate) could be sustained for repeat bouts of 2.5 km. The experimenters gave the subjects verbal encouragement throughout the entire exercise session. Subjects had complete control over the gear ratio, which enabled them to adjust the level of resistance on the bike, similar to what they would experience in training and competition. During exercise, subjects viewed their average and real-time power output, cadence, speed, distance, time, and heart rate on a computer monitor. The face mask was removed immediately after each interval for no more than 30 s so that the subjects could drink water. This ensured that all subjects remained adequately hydrated as determined by collecting pre- and postexercise mass. The remainder of the recovery period was in hypoxic conditions.

**Computed tomography (*day 2*).** Subjects reported to the Department of Radiology at Vancouver General Hospital where they underwent pre- and postexercise limited low-dose CT scans. All subjects underwent a pregnancy test immediately prior to the CT scan to avoid the potential risk of fetal exposure to radiation. CT scans were obtained using a single-turn 360° axial acquisition on a Siemens "Sensation 16," 16 detector row CT scanner (Siemens AG Medical Solutions, Erlangen, Germany) with the subjects in the supine position. The CT parameters were identical to those used in a previous study from our laboratory in male cyclists (26). Briefly, scanner parameters included: 120 kVpeak, 80 mA, 0.5-s rotation time, and a field of view of 380 mm. Automatic tube current modulation was applied in the x,y plane with an average dose length product per acquisition of  $34 \pm 1.4$  mGy/cm yielding an effective dose for the pre- and postexercise scans of 0.95 mSv.

To reduce the potential variability in lung volume between pre- and postexercise CT scans, subjects were instructed to hold their breath at maximal inspiration. Variation in lung CT density is lowest at inspiratory level (34) and breath hold at maximal inspiration is considered to be most reproducible (38). The subjects were trained to perform maximal inspiratory maneuvers and were then asked to execute the maneuver during the CT scan image acquisition (3). Density may also be influenced by the craniocaudal position of the slice through the lung (35) and therefore slice position was based on easily identified non-lung anatomical landmarks that were specified by certified CT scan technologists. Ten 1-mm-thick slices were obtained at the level of the aortic arch, the tracheal carina, and the superior end plate of the tenth thoracic vertebra (T10). Images were reconstructed using a 180° linear interpolation algorithm and an intermediate B45 spatial frequency reconstruction algorithm. The lung parenchyma was segmented and the x-ray attenuation values of the lung were analyzed using custom software (EmphylxJ) as previously described (4, 8). CT slice volume was calculated by summing the voxel dimensions of lung tissue following segmentation from the chest wall and mediastinum. The x-ray attenuation of the lung within each slice was calculated and converted to a CT density value in grams per milliliter using the relationship: density = [lung attenuation [in Hounsfield units (HU)] + 1,000] / 1,000.

CT scans can determine the physical density of the lung parenchyma because the attenuation value of a pixel has a linear relationship to the physical density of the tissue (3). The mean, median, standard deviation, variance, and skewness of the CT density histogram parameters for each CT slice at each anatomic location were calculated to examine potential differences in lung density between subjects.

**Statistical analysis.** Paired *t*-tests were used to determine if there were any differences in pre- or postexercise CT parameters (Statistica 6.1, Stat Soft, Tulsa, OK). The level of significance was set at  $P < 0.05$  for all statistical comparisons. All data are presented as means  $\pm$  SD.

## RESULTS

**Spirometry and  $\dot{V}O_{2max}$ .** Table 1 shows anthropometric and spirometry data obtained on *day 1*. All subjects had normal pulmonary function and had an FEV<sub>1.0</sub>/FVC > 80% of predicted. Table 2 is a summary of data obtained at maximal exercise during the incremental cycle test to exhaustion. Subjects had a maximal aerobic capacity that was 137% of predicted values, indicating that these women were highly trained. Three women had mild exercise-induced arterial hypoxemia (EIAH), three had moderate EIAH, and two did not experience significant EIAH according to previously established criteria (9).

Table 1. Physical characteristics and spirometry

	Value	Range
Age, yr	26 $\pm$ 7	(18–39)
Height, cm	172.2 $\pm$ 6.7	(164–182)
Mass, kg	64.1 $\pm$ 6.7	(54–73)
BMI, kg $\cdot$ m <sup>-2</sup>	21.5 $\pm$ 0.8	(20–22)
FVC, liters	4.3 $\pm$ 0.5	(3.8–5.0)
FVC, %predicted	107.7 $\pm$ 9.0	(95–121)
FEV <sub>1.0</sub> , liters	3.7 $\pm$ 0.4	(3.2–3.7)
FEV <sub>1.0</sub> , %predicted	107.2 $\pm$ 8.2	(99–118)
FEV <sub>1.0</sub> /FVC, %	85.5 $\pm$ 4.0	(79–91)
FEV <sub>1.0</sub> /FVC, %predicted	100.0 $\pm$ 4.6	(95–106)

Values are means  $\pm$  SD. BMI, body mass index; FVC, forced vital capacity; FEV<sub>1.0</sub>, forced expiratory volume in 1 s. Predicted values based on Knudsen et al. (24).

Table 2. Ventilatory, metabolic, saturation and power data at maximum normoxic exercise

	Value	Range
SaO <sub>2</sub> , %	94 $\pm$ 3	(90–98)
$\dot{V}E$ , l/min	109.0 $\pm$ 17.0	(84–140)
VT, liters	1.8 $\pm$ 0.3	(1.7–2.6)
<i>f</i> , breaths/min	60.0 $\pm$ 10.5	(47–76)
$\dot{V}O_2$ , l/min	3.3 $\pm$ 0.4	(2.7–3.9)
$\dot{V}O_2$ , ml $\cdot$ kg <sup>-1</sup> $\cdot$ min <sup>-1</sup>	52.2 $\pm$ 2.2	(49–57)
$\dot{V}O_2$ , %predicted	137 $\pm$ 9	(124–152)
$\dot{V}CO_2$ , l/min	4.2 $\pm$ 0.6	(3.4–5.1)
RER	1.26 $\pm$ 0.03	(1.22–1.31)
HR, beats/min	190 $\pm$ 12	(169–207)
Power, W	335 $\pm$ 33	(278–375)

Values are means  $\pm$  SD. SaO<sub>2</sub>, arterial oxyhemoglobin saturation;  $\dot{V}E$ , minute ventilation; VT, tidal volume; *f*, breathing frequency;  $\dot{V}O_2$ , oxygen consumption;  $\dot{V}CO_2$ , carbon dioxide production; RER, respiratory exchange ratio; HR, heart rate.  $\dot{V}O_2$  prediction equation from Jones et al. (22).

**Hypoxic intervals.** Table 3 summarizes the mean metabolic and exercise performance data during the hypoxic interval exercise. The average combined duration of all intervals was 21.7  $\pm$  0.1 min. The average duration of the entire exercise session, including the warm-up, intervals, and recovery was 61.7  $\pm$  0.06 min. Resting hypoxic SaO<sub>2</sub> was 95  $\pm$  2% and mean SaO<sub>2</sub> during the intervals was 82  $\pm$  4%, resulting in a  $\Delta$ SaO<sub>2</sub> of 13  $\pm$  2%. Subjects exercised at 94.5  $\pm$  2.2% and 81.3  $\pm$  4.9% of their maximum normoxic heart rate and  $\dot{V}E$ , respectively. Figure 1 shows the mean power, heart rate,  $\dot{V}E$ , and SaO<sub>2</sub> response for each 2.5-km interval. There was no change in body mass following exercise, indicating that all subjects remained adequately hydrated throughout the intervals.

CT. Postexercise CT scans were obtained 44  $\pm$  8 min (range 33–62 min) following the completion of the last interval. Table 4 summarizes the total volume, mass, and density of the three regions that were imaged in each individual subject (total slice thickness = 30 mm). There was no change in mean CT slice volume ( $P = 0.38$ ), mass ( $P = 0.28$ ), or CT density ( $P = 0.27$ ) following exercise. Table 5 breaks down the density values into three distinct regions from the apex to the base of the lung in each individual subject. There was no change in density at the level of the aortic arch ( $P = 0.37$ ), the tracheal carina ( $P = 0.25$ ), or the superior end plate of T10 ( $P = 0.24$ ) following exercise. Furthermore, there was no difference in the average median, standard deviation, variance, and skewness of the lung density histograms following exercise ( $P > 0.05$ ), as shown in Table 6.

Table 3. Mean performance, metabolic and ventilatory parameters for all hypoxic intervals

	Value	%Normoxic Max
Time, s	260 $\pm$ 3	
SaO <sub>2</sub> , %	82 $\pm$ 4	
$\dot{V}E$ , l/min	88.3 $\pm$ 11.8	81.3 $\pm$ 4.9
VT, liters	1.7 $\pm$ 0.3	90.8 $\pm$ 5.9
<i>f</i> , breaths/min	54.1 $\pm$ 9.2	90.6 $\pm$ 9.8
$\dot{V}O_2$ , l/min	2.6 $\pm$ 0.5	75.7 $\pm$ 10.2
$\dot{V}CO_2$ , l/min	2.6 $\pm$ 0.4	62.1 $\pm$ 4.5
HR, beats/min	179 $\pm$ 10	94.5 $\pm$ 2.2
Power, W	212 $\pm$ 31	63.3 $\pm$ 5.1

Values are means  $\pm$  SD.



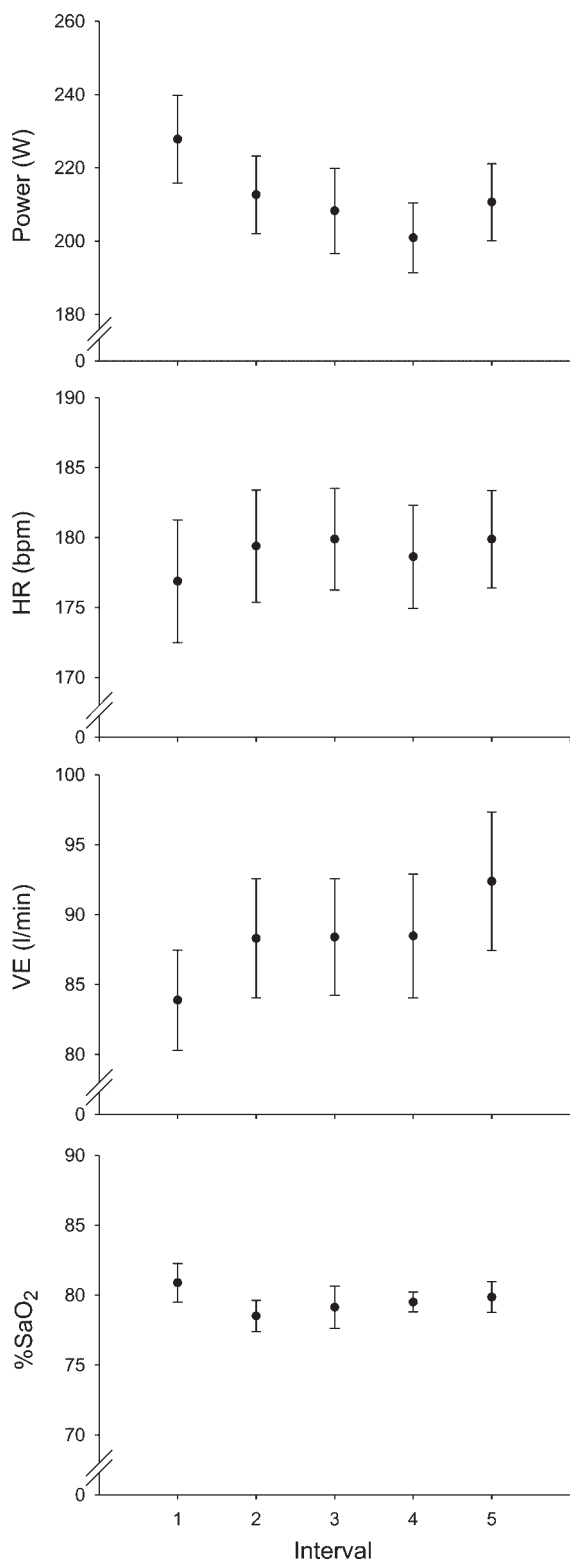


Fig. 1. Mean power output, heart rate (HR), minute ventilation ( $\dot{V}_E$ ) and arterial oxyhemoglobin saturation ( $Sa_{O_2}$ ) for each 2.5-km interval. Values are means  $\pm$  SE.

## DISCUSSION

The purpose of this study was to determine if female cyclists developed pulmonary edema following intense normobaric hypoxic interval training. This is the first study to measure lung

density following hypoxic exercise in trained female athletes. We reasoned that exercise at near maximal intensities coupled with hypoxia-induced vasoconstriction would maximize pulmonary artery pressures and therefore cause disruptions to the blood-gas barrier. However, we did not find evidence of pulmonary edema in any subjects. This study, combined with our previous work in men (26), suggests that pulmonary edema does not develop following intense normobaric hypoxic interval training in either sex.

There have been some anecdotal reports of hemoptysis or tasting blood in humans following intense exercise (42, 46), which may indicate structural damage to the blood-gas barrier. Hopkins et al. (20) showed increased red blood cell counts and protein in bronchoalveolar lavage fluid following 7 min of maximal intensity cycling. Several additional studies corroborate these findings by demonstrating evidence of pulmonary edema following exercise using noninvasive imaging techniques such as x-ray (2) and CT (3). A recent study by McKenzie et al. (30) detected a 9.4% increase in extravascular lung water (EVLW) following a 45-min endurance cycle test using MRI, which was coupled with a decrement in  $DL_{CO}$  and capillary blood volume ( $V_c$ ). Although this statistically significant increase in lung density was primarily driven by two subjects, this may be an underestimation of EVLW in light of the decrease in  $V_c$  from rest. Many additional studies have used similar imaging techniques but have failed to detect any evidence of pulmonary edema following exercise (11, 18, 26, 27, 40). The discrepancy between studies is primarily attributable to methodological discrepancies such as differences in exercise modalities, imaging techniques, and subject fitness levels.

Most studies attempting to examine pulmonary edema following exercise have used male subjects, but there is some evidence to suggest that the response may be different in women. Recent work by Zavorsky et al. (51) found radiographic evidence of pulmonary edema in 9 of 14 fit women following intense sea level cycling interval training. We conducted a similar study to the present investigation in well-trained men (26) using CT in addition to having subjects exercise in hypoxia. Contrary to Zavorsky et al. (51), we were unable to detect any evidence of pulmonary edema. The current study was conducted to determine whether our discrepant results were due to sex differences or differences in imaging methods. After comparing the present study to a methodologically identical study we conducted in men (26), we conclude that there is no sex difference and women are no more likely to develop pulmonary edema following intense interval training than are men. The question then is why did we not show evidence of pulmonary edema in women using a more sensitive imaging technique than used in the study by Zavorsky et al. (51)? Certainly this was not due to insufficient exercise intensity in our study, as the present exercise protocol was more intense than that used by Zavorsky et al. (i.e., 3 $\times$ 5 min "all-out" with 10-min recovery between efforts). Our method of measuring lung density is more sensitive than that used by Zavorsky et al. (51). Our analysis used a computerized objective quantification of lung density, while Zavorsky et al. (51) scored the radiographs using a three-point scale (0, 1, or 2) based on the presence or absence of seven previously established markers of edema. Subjective data can lead to an increase in variability observed in their study and this may

Table 4. Individual mean lung volume, mass, and density pre and postexercise

Subject No.	Volume, ml		Mass, g		Density, g/ml	
	Pre	Post	Pre	Post	Pre	Post
1	867±41	909±29	110±6	111±5	0.1265±0.002	0.1214±0.006
2	654±34	653±49	84±4	83±6	0.1279±0.004	0.1272±0.004
3	853±29	859±33	124±6	123±6	0.1452±0.008	0.1427±0.008
4	693±38	706±29	85±5	91±4	0.1223±0.001	0.1283±0.001
5	881±21	848±29	128±3	122±1	0.1456±0.009	0.1452±0.012
6	856±14	845±15	124±3	126±4	0.1444±0.008	0.1489±0.007
7	723±30	713±33	95±6	94±6	0.1308±0.009	0.1319±0.007
8	776±14	788±25	127±0.3	118±4	0.1645±0.009	0.1501±0.004
Mean±SD	788±89	790±90	110±19	109±17	0.1384±0.014	0.1370±0.011

have contributed to the discrepancy between studies. We are confident that our technique is sensitive enough to detect small changes in lung density because the 95% confidence intervals of the mean density measurements are <10% of mean density. Although we cannot exclude the possibility of a type 2 error in our conclusion because of our relatively small sample size, we are confident that quantitative CT is sensitive enough to measure small changes in lung density due to edema (see CT).

One potential explanation for the discrepancy between this study and the study by Zavorsky et al. (51) comes from the recent work of Snyder et al. (40) that shows a decrease in lung density during normobaric hypoxic ( $F_{I_{O_2}} = 12.5\%$ ) exposures at rest and during exercise in untrained individuals. These authors suggest that hypoxia may reduce lung water by stimulating an increase in lymph flow and/or  $\beta_2$ -adrenergic receptors. Lymph flow removes fluid from the interstitial space and reduces interstitial hydrostatic pressure, which prevents excessive alveolar fluid accumulation (25). Thus it is possible that the normobaric hypoxia-induced increases in the transepithelial pressure gradient were not sufficient to supercede lung fluid clearance mechanisms (40). This may explain, in part, why previous studies using normobaric hypoxia have not shown an increase in lung density following exercise (18, 26, 40), whereas pulmonary edema has been induced by normoxic exercise (3, 30, 51). Interestingly, there is radiographic evidence of edema following prolonged high-intensity exercise in hypobaric hypoxia (~2,400 m; Ref. 2), suggesting a potential effect of pressure, independent of inspired oxygen levels.

**Exercise protocol.** Previous studies on pulmonary edema and exercise have used a wide range of exercise modalities with a variety of intensities, durations, and environmental conditions. A recent review suggests that exercise intensity is the main factor contributing to edema formation during exercise (50). Results of this meta-analysis ( $n = 137$  subjects) showed that 65% of subjects demonstrate signs of edema triggered by maximum effort exercise (defined as maximum or near maximum effort where the goal is to finish the fastest possible time or maintain the highest possible workload), whereas only 16% of subjects show signs of edema following prolonged exercise (defined as 15 min to 2 h at ~50–75% of  $\dot{V}_{O_{2max}}$ ) and 0% from graded exercise tests. The protocol used in this study was specifically designed to maximize both intensity and duration but also to facilitate a comparison with our previous study in men (26) and similar work in women (51). Although we did not measure cardiac output or pulmonary wedge pressures, we are confident that our protocol was sufficient to elevate these variables to near maximal levels. For example, the subjects in the present study sustained  $94.5 \pm 2.2\%$  of their maximum normoxic heart rate for the entire duration of the intervals, including the period of time where heart rate is rising toward steady state. In fact, this value approached 96–97% when only the steady-state heart rate was analyzed. The additional hypoxic intervention caused these cyclists to exercise at an  $Sa_{O_2}$  of  $82 \pm 4\%$ , which was considerably below their resting normoxic and hypoxic  $Sa_{O_2}$  values. Despite the rigorous exercise protocol and additional hypoxic stress, we were unable to detect an increase in lung

Table 5. Individual mean lung density (g/ml) at the aortic arch, tracheal carina, and superior end plate of T10 pre- and postexercise

Subject No.	Aortic Arch		Tracheal Carina		T10	
	Pre	Post	Pre	Post	Pre	Post
1	0.1250±0.003	0.1143±0.002	0.1255±0.003	0.1247±0.007	0.1290±0.003	0.1253±0.003
2	0.1300±0.004	0.1281±0.002	0.1238±0.004	0.1230±0.004	0.1302±0.001	0.1301±0.001
3	0.1414±0.002	0.1417±0.004	0.1397±0.004	0.1356±0.001	0.1543±0.005	0.1508±0.001
4	0.1214±0.001	0.1289±0.002	0.1233±0.006	0.1266±0.002	0.1221±0.002	0.1292±0.002
5	0.1469±0.002	0.1575±0.003	0.1363±0.003	0.1341±0.003	0.1536±0.005	0.1439±0.005
6	0.1397±0.001	0.1446±0.002	0.1401±0.004	0.1452±0.004	0.1532±0.003	0.1569±0.003
7	0.1225±0.002	0.1252±0.002	0.1288±0.006	0.1314±0.004	0.1409±0.003	0.1389±0.003
8	0.1740±0.005	0.1493±0.002	0.1632±0.007	0.1469±0.003	0.1565±0.004	0.1542±0.006
Mean±SD	0.1376±0.018	0.1362±0.014	0.1351±0.013	0.1334±0.009	0.1425±0.014	0.1412±0.012

Values are means ± SD in g/ml. T10, tenth thoracic vertebra.

Table 6. Individual mean values for median, SD, variance, and skewness of the lung density histograms pre- and postexercise

Subject No.	Median, g/ml		Standard Deviation, g/ml		Variance, g/ml		Skewness, g/ml	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	0.087±.003	0.079±0.001	0.159±0.013	0.166±0.015	0.025±0.004	0.028±0.005	3.786±0.063	3.815±0.243
2	0.094±0.005	0.092±0.006	0.144±0.014	0.147±0.016	0.021±0.004	0.022±0.005	3.853±0.199	3.876±0.131
3	0.110±0.002	0.107±0.002	0.149±0.024	0.151±0.021	0.023±0.007	0.023±0.006	3.550±0.183	3.598±0.138
4	0.089±0.004	0.093±0.004	0.143±0.012	0.149±0.012	0.021±0.004	0.022±0.004	3.817±0.042	3.720±0.090
5	0.111±0.003	0.109±0.012	0.152±0.016	0.153±0.017	0.023±0.005	0.024±0.005	3.402±0.059	3.413±0.081
6	0.106±0.004	0.108±0.004	0.155±0.009	0.161±0.009	0.024±0.003	0.026±0.003	3.614±0.206	3.472±0.217
7	0.093±0.004	0.092±0.002	0.159±0.020	0.165±0.013	0.026±0.006	0.027±0.004	3.631±0.123	3.551±0.009
8	0.124±0.013	0.115±0.003	0.162±0.011	0.150±0.015	0.026±0.004	0.023±0.005	3.429±0.165	3.591±0.110
Mean±SD	0.102±0.013	0.099±0.012	0.153±0.015	0.155±0.015	0.024±0.005	0.024±0.005	3.635±0.204	3.618±0.184

density in any subject. To maximize exercise intensity, it was important to give our subjects adequate recovery between each 2.5-km effort. It is possible that the low-intensity active recovery (5 min) may have facilitated edema clearance following each interval. However, subjects were only permitted ~1 min of active recovery following the last interval and were then immediately driven to the hospital for their postexercise CT scan.

**CT.** Diagnostic imaging techniques such as chest radiography, MRI, and CT have been used as noninvasive methods of assessing changes in lung structure in both health (6) and disease, including emphysema (7, 31, 32), pulmonary fibrosis (5, 16, 31), and edema following exercise (3, 18, 26). CT and MRI are thought to be more accurate than chest x-rays because they provide quantitative measures of lung density, whereas chest radiography is based on subjective interpretation of radiographs. Studies using MRI of the lung are limited because of the inherent difficulty in imaging the lung using MRI (i.e., respiratory and cardiac motion, field inhomogeneity issues) and because of the increased expense and time. CT scanning on the other hand has seen a rapid expansion in lung density studies in recent years. For example, Scillia et al. (36) recently investigated the accuracy of CT attenuation measurements for quantifying EVLW in canines and showed that CT indexes became altered immediately after EVLW increased. More importantly, these authors demonstrated significant correlations between the amount of EVLW and measurements of CT density, including mean ( $r = 0.994$ ), median ( $r = 0.986$ ), mode ( $r = 0.989$ ), and standard deviation ( $r = 0.978$ ;  $P < 0.001$ ). We have no way of directly determining the specific volume of EVLW needed to increase lung density in our subjects. However, based on a regression from Scillia et al. (36) relating HU to EVLW, we estimated that 110 ml of EVLW would be needed for us to see a 10% increase in lung density. Although we adjusted for size differences, we acknowledge that this estimation is based on a regression from dogs and generalizing to humans may not be appropriate.

Although CT is an accurate and objective method of assessing pulmonary edema, there are some methodological considerations that warrant discussion. CT-derived lung density may be influenced by a number of factors including technical aspects of the scanner (CT manufacturer, model, reconstruction algorithm, and x-ray dose; Ref. 23) as well as physiological parameters including the level of inspiration and increased pulmonary blood flow during the scan. Indeed, it has been shown that acute increases in

intravascular volume influences pulmonary density in miniature pigs (17). Therefore, any increase in lung density following exercise may simply be a reflection of increased pulmonary circulation rather than edema. To alleviate the technical factors, we performed all CT scans using the same CT scanner and the same technical parameters. To address the issue of lung inflation during the scan, the subjects were coached extensively on how to inhale and suspend their inhalation during the scan. Previous work from our laboratory has shown that female cyclists are able to reproducibly achieve total lung capacity when performing several maximal inspiratory maneuvers (verified with measures of maximal inspiratory esophageal pressure; Ref. 13). We also delayed the second CT scan for 44 min following exercise to allow pulmonary blood flow to return to baseline levels because Manier et al. (28) showed that pulmonary capillary blood volume returns to near resting levels ~30 min following maximal exercise.

Despite improving our time delay by 32 min from our previous study in men (26), it is possible that the women in this study experienced a transient increase in lung water but the time delay allowed for adequate removal. This remains a possibility but it is important to acknowledge that Caillaud et al. (3) detected an increase in lung density using CT following a triathlon race, despite a time delay that was more than twice as long as the time delay in the present study. Therefore, the likelihood of edema removal within our 44-min time delay is unlikely. The difference between the present study and the work of Caillaud et al. (3) may be attributable to differences in exercise protocols (laboratory cycling intervals vs. triathlon race) and/or inspired gas concentration differences (i.e., 15% O<sub>2</sub> vs. normoxia).

Another limitation of our study is that we did not scan the entire lung to avoid excessive radiation exposure to our subjects. Instead, we scanned three anatomical regions corresponding to the superior, inferior, and middle portion of the lungs. These representative regions combined to form a total of 30 mm [(3 regions) × (10×1-mm slices)] of lung tissue or ~18% of total lung volume and this was assumed to be representative of the entire lung. It could be argued that using a limited number of slices may preclude an accurate assessment of lung changes following exercise and full lung reconstruction may be more appropriate (27). However, Caillaud et al. (3) demonstrated an increase in lung density following a triathlon using a limited number of 1-mm-thick slices rather than imaging the entire lung. Lung density in the present study was determined by averaging the density of all three regions.



However, we also performed additional analyses to determine if there was an increase in lung density at a particular region of the lung (Table 5). The data suggests that density was unchanged irrespective of the lung region (i.e., aortic arch, tracheal carina, T10).

**Conclusions.** We found that lung density does not change following intense normobaric hypoxic interval training in female cyclists. Our data contradicts a recent study performed in a similar group of women performing sea level interval training (51). The discrepancy may be attributable to an increase in lymph flow associated with hypoxia. It is possible that the hypoxic-induced increases in pulmonary pressures coupled with extremely intense exercise was not sufficient to interfere with lung fluid clearance mechanisms. However, this study combined with previous work (18, 40) suggests that pulmonary edema does not develop in trained or untrained men and women during normobaric hypoxic exercise. Additional studies are required to determine the role that barometric pressure might play in the development of pulmonary edema following strenuous exercise in healthy humans.

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