Respiratory Physiology & Neurobiology 205 (2015) 99–104

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Does menstrual cycle phase affect lung diffusion capacity during exercise?

Joshua R. Smith*, Kelly R. Brown, Jill D. Murphy, Craig A. Harms

Department of Kinesiology, Kansas State University, 1A Natatorium, Manhattan, KS 66506, USA

ARTICLE INFO

Article history:
Accepted 22 October 2014
Available online 31 October 2014

Keywords:
Lung diffusion capacity
Menstrual cycle
Exercise
Pulmonary blood volume

ABSTRACT

Resting lung diffusing capacity (DLCO) decreases during the early and late-follicular phases of the menstrual cycle presumably due to capillary blood volume (V_C) changes; however, it is not known if these differences exist during exercise. We hypothesized that DLCO would increase during the mid-luteal phase of the menstrual cycle due to increases in V_C. Eight normally menstruating females (21.4 ± 0.7 yrs) were studied. Subjects completed a discontinuous treadmill $\dot{V}O_{2max}$ test during the early-follicular (EF), late-follicular (LF), and mid-luteal (ML) phases of the menstrual cycle. Metabolic measurements were made from a breath-by-breath automated cart, and DLCO via the single-breath exhalation technique during exercise. During exercise, DLCO was lesser during EF compared to ML at 90% and 100%$\dot{V}O_{2max}$ ($p < 0.05$) (90%: 37.8 ± 3.7 EF vs 41.6 ± 4.0 ML, 100%: 37.7 ± 3.7 EF vs 42.6 ± 4.3 ML mL/mmHg/min). V_C was significantly greater during the ML phase when compared to the EF at 80%, 90%, and 100%$\dot{V}O_{2max}$. These results demonstrate DLCO and V_C are influenced by the menstrual cycle during heavy exercise.

1. Introduction

Women have smaller lung volumes, airway diameters, and lower resting lung diffusing capacity when compared to age-, sitting height-, and stature matched males (Mead, 1980; Thurlbeck, 1982; Schwartz et al., 1988), suggesting that women may be more susceptible to developing pulmonary limitations to exercise than men. Because of these morphological sex differences, it has been suggested that women are more likely to exhibit exercise-induced arterial hypoxemia (EIAH) compared to men (Hopkins and Harms, 2004). An unresolved issue is whether pulmonary function is altered during different phases of the menstrual cycle. High estrogen concentration increases fluid retention and therefore increases blood volume (Carlberg et al., 1984). Greater blood volume leads to increased surface area available for pulmonary diffusion. Logically, when estrogen concentration is typically highest during the mid-luteal phase of the menstrual cycle, pulmonary diffusion should be increased. Sansores et al. (1995) showed that resting diffusing capacity (DLCO) is reduced during the early-follicular phase of the menstrual cycle when compared to the late-follicular and mid-luteal phases. The authors speculate that this difference is likely attributed to decreases in pulmonary blood volume. This notion is supported by Seaton (1972) who reported greater pulmonary capillary blood volume during the luteal phase of the menstrual cycle. These effects during exercise have, to date, been untested.

Therefore, the aim of this study was to examine the effects of the menstrual cycle on lung diffusing capacity during exercise in women. Specifically, we hypothesized that during the mid-luteal phase of the menstrual cycle when estrogen is highest, DLCO would be greater compared to the early- and late- follicular phases due to increased pulmonary capillary blood volume.

2. Methods

Eight normally menstruating women volunteered for this study. All subjects completed a medical history questionnaire and signed informed consent prior to participation, which was approved by the Kansas State University Institutional Review Board and in accordance with the *Declaration of Helsinki*. All subjects were non-smokers, free from illness/injury, had no lung or cardiovascular disorders and ranged from sedentary to highly fit (range of $\dot{V}O_{2max}$: 35–53 mL/kg/min). Subjects were interviewed prior to testing as part of the initial screening process to determine menstrual cycle history and current stage of menstrual cycle. Three subjects were currently taking tri-phasic oral contraceptives for at least six months prior to testing to establish a regular menstrual cycle (Beidlemen et al., 1999). Self-report, daily record, and

* Corresponding author. Tel.: +1 785 532 6765; fax: +1 785 577 4098.
E-mail address: smith424@ksu.edu (J.R. Smith).

http://dx.doi.org/10.1016/j.resp.2014.10.014
1569-9048/

progesterone and estradiol hormone assays were used to verify each subject's menstrual cycle phase.

2.1. Exercise sessions

Subjects initially completed standard pulmonary function tests including total lung capacity (TLC), residual volume (RV), and maximum flow-volume loops (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). TLC was determined via the nitrogen washout technique. Subsequently, all subjects completed an incremental treadmill test (Quinton Instrument Company, Model Q65, Seattle, WA) until exhaustion to determine maximal aerobic capacity ($\dot{V}O_{2max}$). After 3–5 min warm up, subjects ran on the treadmill while the speed was held constant at each subject's desired pace (4–8 mph) while the treadmill grade was increased 2% every 75 s until volitional fatigue. Criteria for $\dot{V}O_{2max}$ was a plateau in $\dot{V}O_2$ with increasing workload (<150 mL), RER greater than 1.10, and/or the subject's maximal heart rate (±10% predicted) was achieved. All subjects met these criteria for $\dot{V}O_{2max}$. Breath-by-breath metabolic data was collected for baseline and throughout exercise (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). Heart rate was determined using ECG leads. Arterial oxygen saturation (SpO_2) was determined continuously via a pulse oximeter (Datex-Ohmeda 3900P) attached to the ear lobe that allowed for visual inspection for adequate blood flow.

Following determination of $\dot{V}O_{2max}$, we calculated 40, 60, 80, 90, and 100% of $\dot{V}O_{2max}$ by multiplying $\dot{V}O_{2max}$ by the percent desired and the corresponding speed and percent grade of the treadmill were obtained for each subject. Subsequently, three testing sessions were performed at the same time of day, corresponding to the early-follicular (EF), late-follicular (LF), and mid-luteal (ML) phases of the menstrual cycle. For each testing session, subjects ran for 3 min at each intensity using a discontinuous protocol while breathing both 21%O_2/N_2 balance and 80% O_2/N_2 balance with a 5 min rest period in between each workload. The order of the workloads and the FIO_2 administered were randomized on each testing day. Subjects breathed 100% O_2 before the runs breathing 80%O_2/N_2 to maintain a high alveolar PO_2. Rating of perceived exertion (RPE) and dyspnea were recorded near the end of each stage.

2.2. Determination of lung diffusing capacity (DLCO)

Lung diffusing capacity for carbon monoxide (DLCO) was corrected for [Hb] via the equation: $DLCO_{adj} = DLCO \times ((10.22 + [Hb])/(1.7[Hb]))$ (Crapo et al., 1995). DLCO was determined intrabreath via the single breath exhalation technique at rest and during each exercise stage using a gas mixture of 0.3% CO, 0.3% CH_4, 21% O_2, and N_2 balance (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). The subjects maximally exhaled and then performed a rapid inhalation to total lung capacity followed by a slow, steady exhalation at ~0.5 L/s for >3 s. This method has previously been used to measure lung diffusion capacity in healthy subjects at rest and during light and heavy exercise (Ramage et al., 1987; Huang et al., 1994; Charloux et al., 2010). Since all subjects were non-smokers, the carboxyhemoglobin (COHb) levels were negligible. Additionally, 2 min between tests has previously been shown to eliminate the carbon monoxide gas from the lungs in healthy humans (Ogilvie et al., 1957). Therefore, DLCO was not corrected for COHb back pressure (Sansores et al., 1995). Pulmonary capillary blood volume (V_C) and membrane diffusing capacity (D_m) were determined using the equation established by Roughton and Forster (1957). This determination requires subjects to breathe gas mixtures at two distinct FiO_2's (0.21, 0.80) using the following equation: $1/DL = 1/D_m + 1/\theta V_C$; where: 1/DL is the resistance to gas exchange offered by the sum of the diffusive barriers, $1/D_m$ is the resistance of the alveolar and capillary membranes, θ is the resistance offered by the reaction kinetics and the binding rates of the red blood cell and hemoglobin, and V_C is the pulmonary capillary blood volume. θ was calculated from the equation (Roughton and Forster, 1957) $1/\theta = 0.33 + (0.0057 \times PO_2)$ where PO_2 indicates the mean pulmonary capillary PO_2 (mmHg) (assumed alveolar PO_2—10 mmHg). The coefficient for infinite red-cell permeability (λ = inf) (Roughton and Forster, 1957) was used in the above equation of theta consistent with previous studies (Sansores et al., 1995; Seaton, 1972). The alveolar PO_2 was calculated from the measured O_2 concentration in the alveolar sample used for the DLCO determination. V_C and D_m were then calculated using a least squares linear regression. Assumptions in this measurement include (1) alveolar oxygen tension is between 80 and 650 mmHg and (2) that θ remain unchanged by exercise (Johnson et al., 1960). Using two FIO_2's rather than three may increase variability and risk of error; however, the calculated pulmonary blood volume and membrane diffusion capacity have previously been reported to not be different (Ceridon et al., 2010). We have previously determined in our laboratory that the reproducibility in these measures are good with a coefficient of variation <10% for DLCO and <9% for V_C (Schwartzbeck et al., 2000, MSSE).

To ensure that there were no significant changes in DLCO, V_C, or DM with repeated testing on different days, we also studied five healthy non-smoking men of similar age as our women to serve as control subjects. These subjects had DLCO, V_C, and D_m measurements during a $\dot{V}O_{2max}$ test on three separate days similar in timing to the tests performed on our female subjects using identical protocols.

2.3. Blood collection

A 3-mL blood sample was collected prior to each exercise bout from the antecubital vein into a heparinized vial for hematocrit and hemoglobin determination. A 5-mL vial was also collected for hormonal assays of progesterone (P_4) and estradiol (E_2) (Diagnostic Systems Laboratories, Inc. Webster, TX). Assays followed the standard principle of radioimmunoassay where I^{125} estrogen and I^{125} progesterone were used to determine the concentration of each respective hormone (Yalow and Berson, 1971). Hemoglobin concentration values were determined using a co-oximeter (Radiometer OSM3). Hematocrit values were obtained using a micro-capillary centrifuge. All samples were measured in triplicate with the mean values reported for each cycle phase.

2.4. Statistical analysis

Data are expressed as mean ± standard error. Data were analyzed using SigmaStat statistical software (Jandel Scientific Software). A one-way repeated measure ANOVA was used to determine the differences in measured variables between menstrual cycle phase in each subject. A Tukey's post-hoc test was used to determine where differences existed between mean variables. Pearson product moment correlation coefficients were used to determine relationships. Significance was set at $p < 0.05$.

3. Results

3.1. Subject characteristics

The mean values for subject demographics, resting lung volumes and pulmonary function are shown in Table 1. All subjects had normal lung volumes and lung function as the mean vital capacity (*VC*), total lung capacity (TLC), forced expiratory volume in 1 s (FEV_1) and the ratio of forced expiratory volume and forced vital