J Appl Physiol 109: 35–46, 2010.
First published April 22, 2010; doi:10.1152/japplphysiol.01341.2009.

Sex differences in exercise-induced diaphragmatic fatigue in endurance-trained athletes

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Submitted 1 December 2009; accepted in final form 21 April 2010

Guenette JA, Romer LM, Querido JS, Chua R, Eves ND, Road JD, McKenzie DC, Sheel AW. Sex differences in exercise-induced diaphragmatic fatigue in endurance-trained athletes. *J Appl Physiol* 109: 35–46, 2010. First published April 22, 2010; doi:10.1152/japplphysiol.01341.2009.—There is evidence that female athletes may be more susceptible to exercise-induced arterial hypoxemia and expiratory flow limitation and have greater increases in operational lung volumes during exercise relative to men. These pulmonary limitations may ultimately lead to greater levels of diaphragmatic fatigue in women. Accordingly, the purpose of this study was to determine whether there are sex differences in the prevalence and severity of exercise-induced diaphragmatic fatigue in 38 healthy endurance-trained men ($n = 19$; maximal aerobic capacity = 64.0 ± 1.9 ml·kg^{-1}·min^{-1}) and women ($n = 19$; maximal aerobic capacity = 57.1 ± 1.5 ml·kg^{-1}·min^{-1}). Transdiaphragmatic pressure (Pdi) was calculated as the difference between gastric and esophageal pressures. Inspiratory pressure-time products of the diaphragm and esophagus were calculated as the product of breathing frequency and the Pdi and esophageal pressure time integrals, respectively. Cervical magnetic stimulation was used to measure potentiated Pdi twitches (Pdi,tw) before and 10, 30, and 60 min after a constant-load cycling test performed at 90% of peak work rate until exhaustion. Diaphragm fatigue was considered present if there was a ≥15% reduction in Pdi,tw after exercise. Diaphragm fatigue occurred in 11 of 19 men (58%) and 8 of 19 women (42%). The percent drop in Pdi,tw at 10, 30, and 60 min after exercise in men ($n = 11$) was 30.6 ± 2.3, 20.7 ± 3.2, and 13.3 ± 4.5%, respectively, whereas results in women ($n = 8$) were 21.0 ± 2.1, 11.6 ± 2.9, and 9.7 ± 4.2%, respectively, with sex differences occurring at 10 and 30 min ($P < 0.05$). Men continued to have a reduced contribution of the diaphragm to total inspiratory force output (pressure-time product of the diaphragm/pressure-time product of the esophagus) during exercise, whereas diaphragmatic contribution in women changed very little over time. The findings from this study point to a female diaphragm that is more resistant to fatigue relative to their male counterparts.

breathing; respiratory mechanics; ventilation; gender; cycling

THE EFFECTS OF SKELETAL MUSCLE fatigue on the ability to perform muscular work has been a topic of interest to exercise and muscle physiologists for over a century. Muscle fatigue can be defined as a loss in the capacity for developing force and/or velocity resulting from muscle activity under load and that is reversed by rest (37). Studies examining sex differences in peripheral skeletal muscle fatigue have shown that women have greater fatigue resistance than their male counterparts (7, 32, 36, 56). Commonly cited mechanisms associated with greater fatigue resistance in women include differences in muscle mass/morphology, substrate utilization, and neuromuscular activation (23). The majority of studies examining sex differences in skeletal muscle fatigue have focused on the muscles involved in moving the elbow, finger, knee, thumb, ankle, back, and neck. To our knowledge, no study has systematically assessed sex differences in fatigue of the human diaphragm.

The diaphragm is embryologically, morphologically, and functionally a striated skeletal muscle. However, it remains distinct from other skeletal muscles because it is under both voluntary and involuntary control and, like the heart, contracts rhythmically across the entire lifespan. Although it appears that nonrespiratory skeletal muscles are more fatigue resistant in women, the diaphragm may be an exception due to known sex differences in respiratory anatomy, which may predispose women to pulmonary limitations during exercise. For example, women have smaller lung volumes and a decreased capacity for lung diffusion even when corrected for age and height (33, 35, 42). Women also have smaller diameter airways even when matched for lung volume (31, 46). These sex differences may explain, in part, why trained women have a higher work of breathing relative to men during exercise (14, 17) and why they may be more susceptible to exercise-induced arterial hypoxemia (15, 39) and expiratory flow limitation (17, 33). On the basis of the aforementioned sex differences, the respiratory muscles of exercising women may be placed under greater mechanical stress and thus may be more likely to develop fatigue. Alternatively, the repetitive exposure to high levels of respiratory work in trained women during exercise may result in respiratory muscles that have adapted to resist fatigue.

Previous work from our laboratory (17, 45) and others (19) have postulated that these anatomic and functional sex differences in pulmonary physiology might make the female diaphragm more prone to fatigue. This hypothesis is contradictory to previous studies showing that women may be less susceptible to fatigue of the limb muscles (23, 24) and perhaps even cardiac muscle (43). Therefore, the purpose of this study was to determine whether there are sex differences in the prevalence and severity of exercise-induced diaphragmatic fatigue. We hypothesized that the prevalence and severity of diaphragm fatigue after high-intensity cycling exercise would be greater in trained women than in trained men.

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METHODS

Subjects

Thirty-eight (19 men and 19 women) healthy subjects gave informed written consent. Thirty-one subjects were competitive endurance-trained cyclists (16 men and 15 women), whereas the remaining 7 subjects were noncompetitive endurance-trained individuals (3 men and 4 women). "Competitive" was defined as regular participation in cycling and/or triathlon races. All of the women were tested randomly throughout the menstrual cycle. Subjects were excluded from participating if they were smokers or had a history of cardiopulmonary disease. Subjects with nasal septum deviation, esophageal ulcers, or allergies to local anesthetics or latex were also excluded from participation. All procedures received institutional ethical approval and conformed to the Declaration of Helsinki.

Experimental Overview

The experiment was conducted on 2 separate days with a minimum of 48 h rest between each testing session. On *day 1*, subjects underwent basic anthropometric measures followed by pulmonary function testing, general experimental familiarization, and an incremental cycle test to exhaustion. *Day 2* served as the primary testing day, which involved the assessment of diaphragmatic fatigue using cervical magnetic stimulation in response to constant load cycling.

Pulmonary Function

We measured forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1/FVC, peak expiratory flow, and forced expiratory flow between 25% and 75% of FVC using a portable spirometer (Spirolab II, Medical International Research, Vancouver, BC) according to ATS/ERS guidelines (1). Pulmonary function variables are reported in absolute values and also as a percentage of predicted values (38). Subjects with an FEV_1/FVC $<$80% of predicted were excluded from participating in the investigation.

Incremental Exercise Test

Subjects performed a 10-min warm-up at a self-selected work rate on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Men and women started the test at 200 and 100 W, respectively, with the work rate increasing in a stepwise fashion by 30 W every 3 min. The test was terminated if the subject's cadence dropped below 60 rpm, despite verbal encouragement from the experimenters. Peak work rate was calculated as the sum of the final completed exercise stage and an extrapolated work rate depending on the time spent in the final uncompleted stage. To determine maximal O_2 consumption, subjects wore a nose clip and breathed through a mouthpiece connected to a nonrebreathing valve (model 2700B, Hans-Rudolph, Kansas City, MO). Mixed expired gases were measured using calibrated CO_2 and O_2 analyzers (models CD-3A and S-3-A/I, respectively, Applied Electrochemistry, Pittsburgh, PA) measured at a port located in a mixing chamber, whereas inspiratory flow was measured with a calibrated pneumotachograph (model 3813, Hans Rudolph). Maximal ventilatory and metabolic parameters were taken as the highest 30-s average. Heart rate was recorded every 30 s with a commercially available heart rate monitor (S610i, Polar Electro, Kempele, Finland).

Time to Exhaustion Test

On *day 2*, subjects performed a 10-min self-selected warm-up on the same cycle ergometer as used on *day 1*. Subjects then exercised at 90% of their previously determined peak work rate for as long as possible until their cadence dropped below 60 rpm. Subjects received continual verbal encouragement throughout the exercise test. Subjects were instrumented with a finger pulse oximeter (Nonin 8600, Nonin Medical, Plymouth, MN) and heart rate monitor (S620i, Polar Electro) to measure arterial oxyhemoglobin saturation and heart rate, respectively, during exercise. Perceptual responses and respiratory flow and pressures were monitored during the time to exhaustion (TTE) test.

Symptom Evaluation

Subjects were asked to rate their overall feeling of breathing and leg discomfort each minute during exercise using Borg's 0–10 category ratio scale (5). The scale's endpoints were anchored such that "0" represented "no respiratory (or leg) discomfort" and "10" represented "the most severe respiratory (or leg) discomfort you have ever experienced or could ever imagine experiencing."

Flow and Pressure Measurements

Subjects breathed through a mouthpiece connected to a bidirectional heated pneumotachograph (model 3813, Hans Rudolph) to continuously measure inspiratory and expiratory air flow. The flow was then integrated to obtain volume. Mouth pressure was monitored at a port located in the mouthpiece and connected to a piezoelectric pressure transducer (Raytech Instruments, Vancouver, BC). Esophageal pressure (Pes) and gastric pressure were measured using balloon-tipped catheters (no. 47–9005, Ackrad Laboratory, Cranford, NJ) attached to piezoelectric pressure transducers (Raytech Instruments). The transducers were calibrated across the physiological range using a digital manometer (2021P, Digitron, Torquay, UK). Viscous lidocaine (2%) was applied to the nasal and pharyngeal passages to minimize discomfort during catheter placement. Both catheters were first inserted into the stomach. The esophageal catheter was withdrawn until a negative pressure deflection was observed during inspiration and then withdrawn an additional 10 cm to ensure that it was completely within the esophagus. After the balloons were inserted, all air was evacuated by subjects performing a Valsalva maneuver. One and two ml of air were injected into the esophageal and gastric balloons, respectively. We verified the validity of the esophageal balloon position using the occlusion technique (4). Transdiaphragmatic pressure (Pdi) was obtained online as the difference between Pes and gastric pressure.

Diaphragm Fatigue

The cervical magnetic stimulation technique was used to assess diaphragmatic fatigue after exercise (47). Both phrenic nerves were stimulated using a 90-mm circular coil attached to a magnetic stimulator (Magstim 200 Mono Pulse, MagStim, Whitland, Wales). The subject was seated in a chair and instructed to flex their neck, and then the coil was placed over the cervical spine. The site of optimal stimulation was determined by positioning the coil between C_5 and C_7 until the largest Pdi was achieved. The position of the coil was then marked with indelible ink on the subject's neck to ensure the coil was in an identical position for all subsequent stimulations. Considerable care was also taken to ensure that subjects were seated in the same position throughout the experiment with the same level of neck flexion. Because lung volume can influence twitch amplitude, all twitches were performed at end-expiratory lung volume (EELV) with the glottis closed. EELV was verified by examining end-expiratory Pes before stimulation. Supramaximal stimulation was assessed by charging the stimulator to progressively increasing levels of its maximal power output (i.e., 50, 60, 70, 80, 85, 90, 95, and 100%). Three twitches were performed at each power output with each twitch separated by 30 s of quiet breathing to avoid twitch potentiation. A plateau in mean twitch Pdi (Pdi,tw) with increasing power output was an indication that the phrenic nerves were maximally stimulated. Surface EMG results of the right and left costal diaphragm were recorded using surface electrodes (Soft-E H59P: Kendall-LTP, Chicopee, MA). The electrodes were placed on the anterior axillary line on the sixth to eighth intercostal spaces and repositioned if necessary to optimize M-wave characteristics (9). Peak-to-peak amplitudes of

the M waves were measured for every twitch. EMG signals were amplified and band-pass filtered, and the analog signals were A/D converted (PowerLab/16SP model ML 795, ADInstruments, Colorado Springs, CO) and recorded simultaneously using PowerLab data-acquisition software (Chart v6.1.3, ADInstruments). Pressure and EMG signals were sampled at 1 kHz.

The protocol for this experiment included a series of five potentiated twitches that were performed at 100% of the stimulator output before (baseline) and 10, 30, and 60 min after the exercise bout. Potentiated twitches involved a maximal inspiration for ~5 s initiated at functional residual capacity against a device that incorporated a 2-mm orifice to prevent glottic closure. The phrenic nerves were then stimulated at the end of the second tidal expiration after the maximal inspiratory effort. The subject then immediately repeated this procedure a total of five times (Fig. 1). We discarded the first two twitches from the analysis because twitch amplitude was still rising. Individual twitches were rejected if there was evidence from the raw Pes trace of deviation from relaxed functional residual capacity or esophageal contraction. The primary outcome variable for this study was the change in Pdi,tw. Additional fatigue and contractility characteristics of the diaphragm included contraction time and half-relaxation time. Contraction time was determined as the time interval between the initiation of twitch tension and peak tension, and half-relaxation time was determined as the time for Pdi to decrease to one-half of the peak tension (Fig. 1). Fatigue of the diaphragm was considered present if there was a ≥15% reduction in Pdi,tw relative to the preexercise baseline values at any time point after exercise as used previously by others (27). This conservative definition of fatigue is based on an approximate two- to threefold increase in the coefficient of variation of Pdi,tw as observed in the present study.

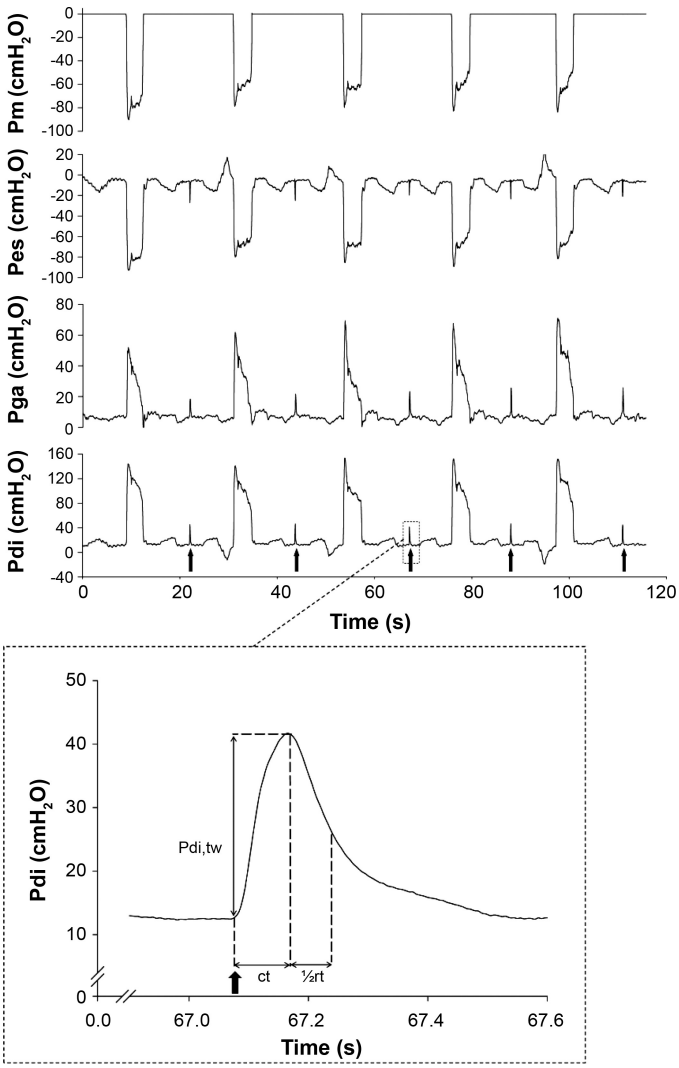

Fig. 1. Twitch potentiation protocol. Example of the twitch potentiation protocol in an individual subject. Each of the 5 maximal inspiratory efforts is followed by a twitch performed at end-expiratory lung volume. *Top*: mouth pressure (Pm), esophageal pressure (Pes), gastric pressure (Pga), and transdiaphragmatic pressure (Pdi). *Bottom*: example of an individual Pdi twitch (Pdi,tw) using cervical magnetic stimulation to stimulate the phrenic nerves. ct, Contraction time; 1/2rt, half-relaxation time; thick vertical arrow represents the onset of cervical magnetic stimulation.

Data Analysis

Approximately 10 consecutive breaths during exercise were selected at various percentages of TTE and ensemble averaged using customized software. Nonrepresentative breaths (i.e., when the subject speaks, coughs, sighs, swallows, etc.) were not included in the breath selection. The work of breathing against the lung was estimated from the area within the tidal Pes-volume loop with the addition of that portion of a triangle describing the work that fell outside the tidal Pes-volume loop representing part of the elastic work of breathing (34). The work of breathing was then multiplied by breathing frequency and converted into joules per minute. Diaphragmatic pressure-time product (PTPdi) and esophageal pressure-time product (PTPes) were determined by integrating Pdi and Pes, respectively, during inspiration with respect to time and then multiplying these values by breathing frequency.

Statistical Analysis

Descriptive characteristics, pulmonary function, and maximal exercise data were compared between groups with unpaired *t*-tests. Preplanned comparisons were used to determine sex differences at each time point during the constant-load cycle test using unpaired *t*-tests with Bonferroni corrections for multiple comparisons. When five comparisons were made, a P value of <0.01 was considered statistically significant and a P value of <0.017 was considered statistically significant when three comparisons were made. P values ≤ 0.05 are also reported throughout the manuscript. One-way repeated-measures ANOVA with a Dunnet post hoc test was used to determine whether there were significant differences in Pdi,tw at all submaximal stimulation intensities compared with maximal stimulation intensity (100%). Linear regression analysis was performed to determine the relationship between diaphragm fatigue and selected exercise parameters. Values are presented as means ± SE.

RESULTS

Subject Characteristics and Pulmonary Function

Table 1 summarizes basic descriptive characteristics and pulmonary function data. Men and women were not different for age, but men were taller and heavier. As expected, men had larger absolute values for FVC, FEV_1, peak expiratory flow, and forced expiratory flow between 25% and 75% of FVC, but there were no sex differences in percent predicted values for any of these parameters.

Incremental Exercise Test

Table 2 summarizes maximal exercise data for men and women on *day 1*. Men had a higher absolute and relative maximal O_2 consumption and higher peak work rates than women. The larger minute ventilation in men was due exclusively to a higher tidal volume. At peak exercise, women reported lower levels of breathing and leg discomfort.

Table 1. *Descriptive characteristics of the subjects*

	Men	Women
Age, years	27 ± 1	28 ± 1
Height, cm	182 ± 2	167 ± 2*
Mass, kg	77 ± 2	62 ± 2*
BMI, kg/m^2	23.3 ± 0.6	22.3 ± 0.4
BSA, m^2	1.98 ± 0.03	1.69 ± 0.03*
FVC, liters	6.0 ± 0.2	4.2 ± 0.1*
FVC, %predicted	110 ± 3	113 ± 2
FEV_1, liters	4.9 ± 0.1	3.7 ± 0.1*
FEV_1, %predicted	107 ± 2	111 ± 2
FEV_1/FVC, %	81.8 ± 1.6	86.2 ± 1*
FEV_1/FVC, %predicted	99 ± 2	103 ± 1
PEF, l/s	11.3 ± 0.3	8.1 ± 0.3*
PEF, %predicted	111 ± 2	112 ± 4
$FEF_{25–75\%}$, l/s	5.0 ± 0.3	4.4 ± 0.3*
$FEF_{25–75\%}$, %predicted	98 ± 5	108 ± 7
MIP, cmH_2O	−119 ± 5	−94 ± 4*

Values are means ± SE. BMI, body mass index; BSA, body surface area; FVC, forced vital capacity; FEV_1, forced expired volume in 1 s; PEF, peak expiratory flow; $FEF_{25–75\%}$, forced expiratory flow between 25 and 75% of FVC; MIP, maximal inspiratory pressure. Prediction equations are from Quanjer et al. (38). *Significant difference ($P < 0.05$) between men and women.

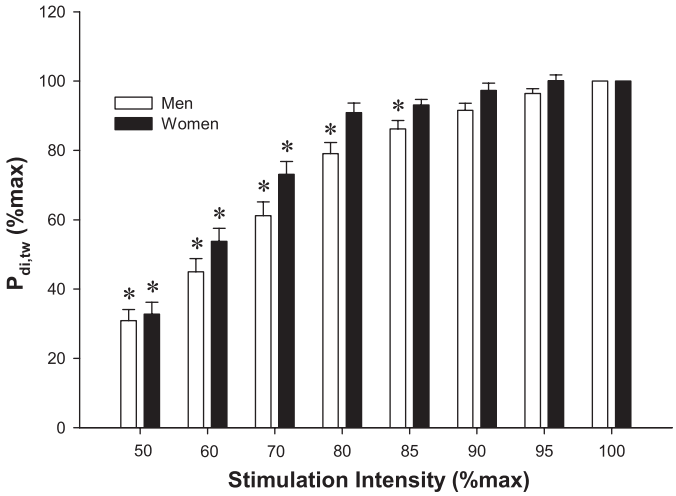

Fig. 2. Pdi response to increasing cervical magnetic stimulation intensities in men and women. Values are means ± SE. *Significantly different ($P < 0.05$) from 100% stimulation intensity.

Supramaximal Stimulation

There was a proportional increase in Pdi,tw as the power output of the stimulator increased to 85–90% and then began reaching a plateau thereafter in men and women (Fig. 2). Clear evidence of a plateau was observed in all of the female subjects and in 11 of the male subjects.

Diaphragm Fatigue

Diaphragm fatigue was present in 11 of 19 males (58%) and 8 of 19 females (42%). Figure 3*A* shows the reduction in potentiated Pdi,tw in all men and women. The percent drop in Pdi,tw at 10, 30, and 60 min following exercise in men ($n = 19$) was 20.4 ± 3.3, 12.0 ± 3.1 and 8.2 ± 3.2%, respectively. The drop in Pdi,tw in women ($n = 19$) at the same time points was 13.0 ± 2.3, 6.7 ± 2.2 and 4.5 ± 2.6% respectively. Figure 3*B* shows the reduction in potentiated Pdi,tw in those subjects who developed diaphragm fatigue as defined by a ≥15% reduction in Pdi,tw. The percent drop in Pdi,tw at 10, 30, and 60 min after exercise in these men was 30.6 ± 2.3, 20.7 ± 3.2, and 13.3 ± 4.5%, respectively. The drop in Pdi,tw in the women that developed fatigue at the same time points was 21.0 ± 2.1, 11.6 ± 2.9, and 9.7 ± 4.2%, respectively. Men consistently had greater reductions in Pdi,tw than women, with the largest differences seen at 10 min postexercise. The absolute drop in Pdi,tw at 10 min postexercise in all men ($n = 19$) and women ($n = 19$) was 8.3 ± 1.3 cmH_2O and 5.2 ± 0.8 cmH_2O ($P < 0.05$), respectively. The absolute drop in Pdi,tw in the men with fatigue ($n = 11$) and the women with fatigue ($n = 8$) was 12.2 ± 0.9 cmH_2O and 8.0 ± 0.7 cmH_2O ($P < 0.01$), respectively. There were no differences in Pdi,tw for the men and women who did not develop diaphragm fatigue (Fig. 3*C*). The average Pdi during the potentiation efforts at 10, 30, and 60 min was 7%, 4%, and 3% lower relative to baseline values in men, respectively, and 3%, 1%, and 3% lower in women, respectively, with no difference between sexes ($P > 0.05$). This suggests that the level of potentiation was the same between men and women. The data presented in Fig. 3 point to a female diaphragm that has greater resistance to exercise-induced diaphragmatic fatigue. Individual Pdi,tw responses at 10, 30, and 60 min after exercise in male and female fatiguers and nonfatigues are shown in Fig. 4, and descriptive characteristics of these groups are shown in Table 3. Peak-to-peak M-wave amplitudes for the right and left sides of the diaphragm at 10, 30, and 60 min postexercise were not different relative to baseline values in men or women (see also Fig. 5). Mean coefficient of variation in Pdi,tw at baseline and at 10, 30, and 60 min postexercise was 5.5, 5.9, 5.5, and 5.5% in men ($P > 0.05$) and 6.7, 5.8, 5.9, and 6.5% in women, respectively ($P > 0.05$), with no differences between sexes. There were no significant sex differences for diaphragmatic contraction time or half-relaxation time at any time point after exercise.

Table 2. *Maximal incremental exercise data on day 1*

	Men	Women
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	64.0 ± 1.9	57.1 ± 1.5*
$\dot{V}O$, l/min	4.9 ± 0.1	3.5 ± 0.1*
$\dot{V}CO_2$, l/min	5.4 ± 0.1	3.8 ± 0.1*
RER	1.12 ± 0.01	1.10 ± 0.01
$\dot{V}E$, l/min	154.7 ± 3.9	109.4 ± 3*
Fb, breaths/min	62.0 ± 2.4	62.7 ± 2.3
VT, liters	3.2 ± 0.1	2.2 ± 0.1*
Heart rate, beats/min	190 ± 2	185 ± 2
Work rate, W	364 ± 10	269 ± 9*
Breathing discomfort, points	9.1 ± 0.2	8.2 ± 0.4*
Leg discomfort, points	9.3 ± 0.2	8.5 ± 0.3*

Values are means ± SE. $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; $\dot{V}E$, minute ventilation; Fb, breathing frequency; Vt, tidal volume; Breathing and leg discomfort are measured in "points" using the modified Borg scale. *Significant difference ($P < 0.05$) between men and women.

TTE Test

Duration, heart rate, and saturation. Work rate for the TTE test in men and women was 327 ± 9 W and 242 ± 8 W, respectively, which corresponded to 90% of their peak work rate as determined on *day 1*. Men cycled for 13.7 ± 0.9 min (range: 9.3–22.9 min), whereas women cycled for 11.4 ± 0.7

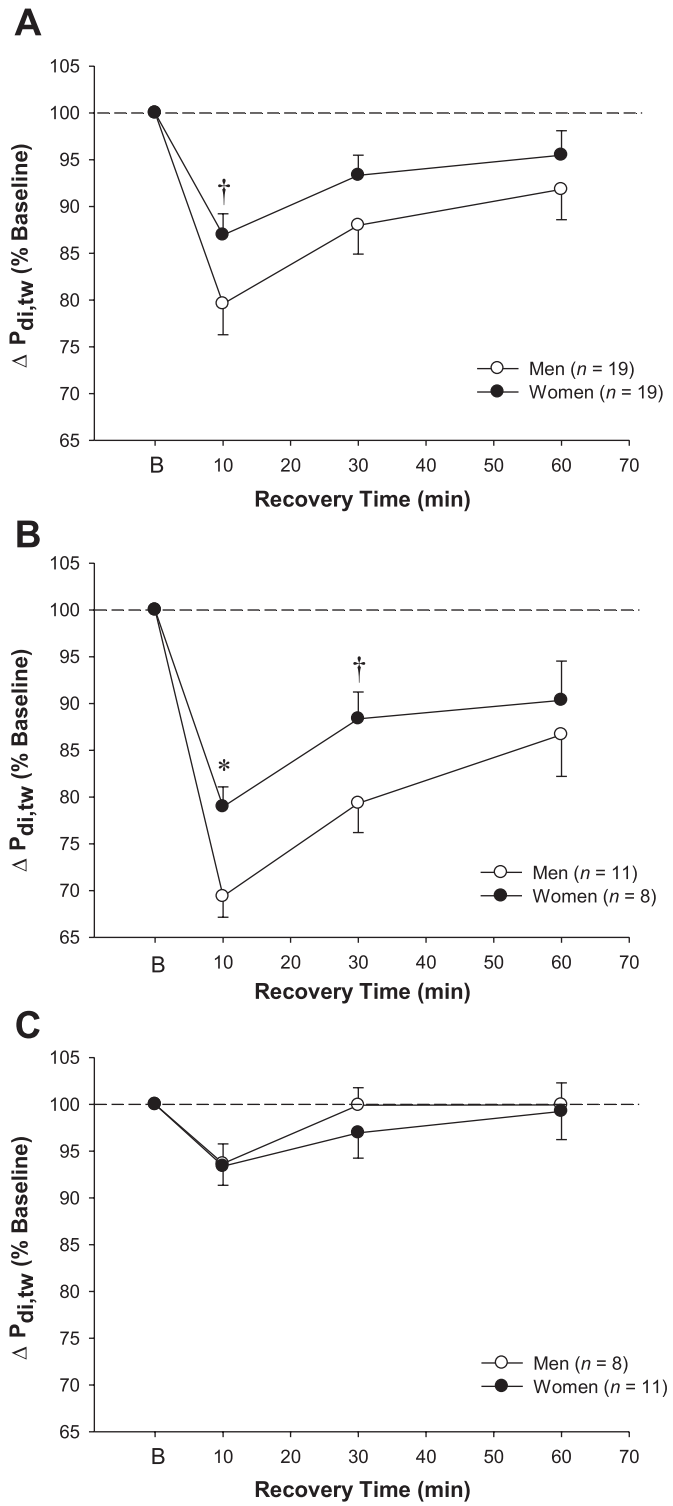

Fig. 3. Response of Pdi,tw during recovery in men and women. Values are means ± SE. *A*: mean response in all men and women. *B*: mean response in the men and women who developed diaphragmatic fatigue (defined as a drop in Pdi,tw of ≥15%). *C*: mean response for the men and women who did not develop diaphragmatic fatigue. Dashed line represents the baseline. Significant differences between men and women are shown (*$P < 0.01$; †$P < 0.05$).

min (range: 7.8–18.0 min) ($P = 0.051$). The exercise duration in the men with fatigue was not different from the men without fatigue (14.1 ± 1.0 vs. 13.0 ± 1.5 min, respectively; $P > 0.05$). Similarly, there was no difference in exercise duration in the women with fatigue compared with those without fatigue (10.9 ± 1.2 vs. 11.8 ± 0.9 min, respectively; $P > 0.05$). For any given time (expressed as a percentage of TTE), women consistently cycled at a higher percentage of their maximum heart rate. During the latter half of the test, men averaged 95% and women averaged 97% of their maximum heart rate ($P < 0.01$). There was no significant sex difference in arterial oxyhemoglobin saturation at exhaustion in men and women (92.6 ± 0.7% vs. 92.3 ± 0.8%, respectively).

Perceptual responses to exercise. Figure 6 shows the ratings of leg and breathing discomfort for men and women during the TTE test. There were no significant differences in perceived exertion, despite women reporting lower levels of breathing and leg discomfort at maximal exercise during the incremental test on *day 1* (Table 2).

Ventilation and breathing mechanics during exercise. Breathing frequency, tidal volume, minute ventilation, minute ventilation expressed as a percentage of maximum minute ventilation (as determined on *day 1*), and the work of breathing are shown in Fig. 7. Women relied on a higher breathing frequency during the first 60% of the exercise test, with no sex differences shown over the last 40% of the test (Fig. 7*A*). Men consistently had higher tidal volumes (Fig. 7*B*) and ventilations (Fig. 7*C*). Women utilized a larger fraction of their maximum ventilation during the first 40% of the exercise test but utilized a lower fraction at the end of the exercise test relative to men (Fig. 7*D*). The minute ventilation-to-maximum ventilation ratio at the end of the exercise test (i.e., 100% TTE) was significantly correlated with the magnitude of diaphragm fatigue in all subjects ($r = 0.52$, $P < 0.001$). Thus those utilizing the largest fraction of their exercise ventilatory capacity at the end of the exercise test tended to demonstrate the greatest diaphragmatic fatigue. The mechanical work of breathing was consistently higher in men throughout the entire exercise test and rose disproportionally compared with women with increasing time (Fig. 7*E*). The absolute- and mass-corrected PTPdi and PTPes are shown in Fig. 8. Men consistently had higher absolute values for PTPdi (Fig. 8*A*). Women had a relative plateau in PTPdi, whereas men continued to increase PTPdi toward exhaustion. Taking mass into account normalizes the PTPdi response such that there is no significant sex difference across time (Fig. 8*B*). There were no differences in absolute PTPes at any time point until exhaustion where PTPes rose disproportionally in men relative to women (Fig. 8*C*). However, the nonsignificant sex difference in PTPes during the majority of the test was reversed when body mass was accounted for (Fig. 8*D*). The PTPes normalized to body mass was, on average, ~15% higher in women across the entire TTE test. The relative contribution of the diaphragm to total inspiratory muscle force output (PTPdi/PTPes) was higher in men at the start of the cycle test, with the data converging once exhaustion was reached (Fig. 8*E*). However, over time, men continued to have a reduced contribution of the diaphragm to total inspiratory force output, whereas diaphragmatic contribution in women changed very little over time. There was a 12% reduction in PTPdi/PTPes from 20% TTE to 100% TTE in men and only a 3% reduction in women. To further characterize this response, mean slopes were calculated across the entire duration of exercise for all subjects (Fig. 8*F*). The mean

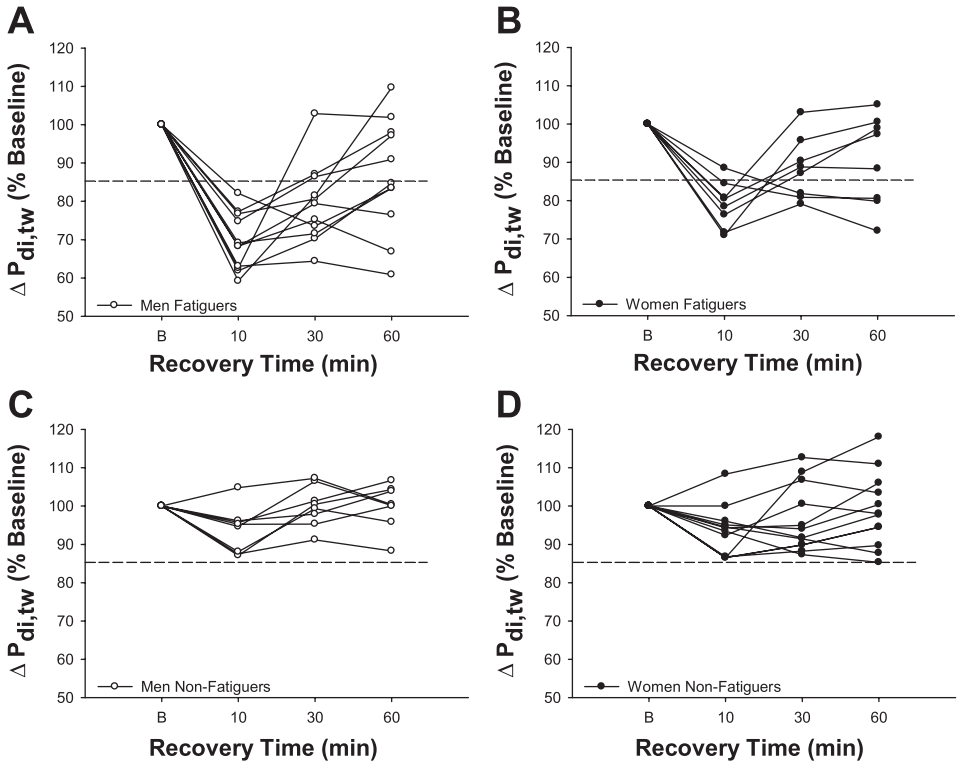

Fig. 4. Pdi,tw during recovery in individual subjects (*A–D*) who did and did not develop diaphragmatic fatigue. Dashed line represents the fatigue threshold of 15%. Note that the 1 woman above threshold in *B* was considered a fatiguer because her Pdi,tw was <15% at the 30-min time point.

slope for men was significantly greater than for women ($P < 0.05$), suggesting a progressive decrease in diaphragmatic pressure contribution during exercise and an increase in accessory muscle recruitment to generate the increasing levels of ventilation shown in Fig. 7*C*. Regression analysis on the mean values relating PTPdi/PTPes to TTE (%max) showed a significant association for men ($r^2 = 0.89$; $P < 0.001$) but not for women ($r^2 = 0.37$; $P > 0.05$).

Table 3. *Descriptive characteristics and maximal exercise data in male and female fatiguers and nonfatiguers*

	Male Fatiguers ($n = 11$)	Male Nonfatiguers ($n = 8$)	Female Fatiguers ($n = 8$)	Female Nonfatiguers ($n = 11$)
Age, years	29 ± 1	25 ± 2	27 ± 2	29 ± 1
Height, cm	185 ± 2	179 ± 3	167 ± 2	166 ± 2
Mass, kg	80 ± 3	74 ± 2	62 ± 3	61 ± 2
BMI, kg/m^2	23.3 ± 0.7	23.3 ± 1.0	22.3 ± 0.9	22.2 ± 0.3
BSA, m^2	2.03 ± 0.04	1.92 ± 0.03	1.70 ± 0.04	1.68 ± 0.04
FVC, liters	6.0 ± 0.2	6.0 ± 0.2	4.3 ± 0.2	4.2 ± 0.2
FVC, %predicted	108 ± 3	113 ± 4	113 ± 4	113 ± 2
FEV_1, liters	4.8 ± 0.2	4.9 ± 0.2	3.7 ± 0.1	3.6 ± 0.2
FEV_1, %predicted	105 ± 3	110 ± 2	110 ± 3	111 ± 3
FEV_1/FVC, %	81.0 ± 2.0	83.0 ± 2.5	86.1 ± 1.7	86.2 ± 1.2
FEV_1/FVC, %predicted	99 ± 2	100 ± 3	102 ± 2	103 ± 1
PEF, l/s	11.5 ± 0.3	11.0 ± 0.5	8.1 ± 0.3	8.1 ± 0.4
PEF, %predicted	112 ± 2	109 ± 4	111 ± 5	113 ± 5
$FEF_{25–75\%}$, l/s	4.8 ± 0.3	5.3 ± 0.4	4.1 ± 0.2	4.5 ± 0.4
$FEF_{25–75\%}$, %predicted	94 ± 6	103 ± 7	100 ± 5	114 ± 11
MIP, cmH_2O	−117 ± 7	−121 ± 7	−97 ± 4.5	−92 ± 5.0
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	61.8 ± 1.8	67.1 ± 3.5	58.3 ± 1.9	56.2 ± 2.1
$\dot{V}O_2$, l/min	4.9 ± 0.2	4.9 ± 0.3	3.6 ± 0.1	3.5 ± 0.2
$\dot{V}CO_2$, l/min	5.4 ± 0.2	5.3 ± 0.2	3.9 ± 0.1	3.7 ± 0.2
RER	1.12 ± 0.01	1.11 ± 0.02	1.10 ± 0.01	1.10 ± 0.01
$\dot{V}E$, l/min	154.5 ± 4.1	155.0 ± 7.6	111.0 ± 3.5	108 ± 4.6
Fb, breaths/min	59.9 ± 3.3	64.9 ± 3.5	64.8 ± 4.4	61.1 ± 2.5
VT, liters	3.3 ± 0.1	3.1 ± 0.2	2.2 ± 0.1	2.2 ± 0.1
Heart rate, beats/min	189 ± 3	191 ± 2	184 ± 4	186 ± 3
Work rate, W	367 ± 14	359 ± 13	280 ± 13	261 ± 12
Breathing discomfort, points	9.4 ± 0.3	8.8 ± 0.4	8.3 ± 0.6	8.1 ± 0.4
Leg discomfort, points	9.6 ± 0.2	8.8 ± 0.3*	8.4 ± 0.6	8.6 ± 0.2

Values are means ± SE. Breathing and leg discomfort are measured in "points" using the modified Borg scale. Prediction equations for pulmonary function are from Quanjer et al. (38). *Significant difference ($P < 0.05$) between fatiguer and nonfatiguer of the same sex.

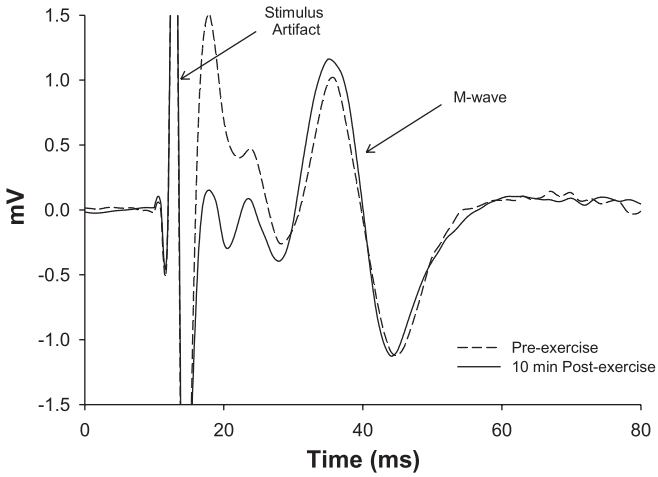

Fig. 5. M-wave example in an individual female subject at baseline and at 10 min postexercise.

DISCUSSION

The purpose of this study was to determine whether there are sex differences in the prevalence and severity of exercise-induced diaphragmatic fatigue in healthy trained men and women. Our findings do not support our original hypothesis that women would develop more diaphragmatic fatigue than men. We have shown that fewer women developed diaphragmatic fatigue and that the magnitude of fatigue was significantly greater in men. To our knowledge, this is the first study to measure Pdi during exercise in a large group of women and the first to assess diaphragm fatigue in women using phrenic nerve stimulation.

Diaphragm Fatigue

The reduction in Pdi,tw 10 min after exercise and the pattern of recovery at 30 and 60 min in our male subjects is consistent with previous studies (2, 3, 52, 54). The time course of Pdi,tw in response to phrenic nerve stimulation was nearly identical between men and women (Fig. 3). That is, the greatest reductions in Pdi,tw were seen 10 min after exercise, with Pdi,tw approaching baseline levels at 60 min. However, the percent drop in Pdi,tw was less in women, particularly at 10 and 30 min after exercise, suggesting greater fatigue resistance in women. The literature concerning the prevalence of exercise-induced diaphragmatic fatigue is controversial due to the wide range of methodologies used, the large variation in subject characteristics, the relatively small sample sizes used, and because some groups define fatigue based on different percent reductions in Pdi,tw. In the present study, we show that the fatigue response of the diaphragm is variable with only about one-half of the subjects showing evidence of diaphragm fatigue (as defined by a $\geq$15% reduction in Pdi,tw). If we had defined fatigue as a $\geq$10% reduction in Pdi,tw as used by others (30, 50), we would have observed fatigue in 14 of 19 men (74%) and 11 of 19 women (58%). It is difficult to determine why some subjects in a relatively homogenous population develop diaphragm fatigue while others do not. There were no remarkable differences in subject characteristics that might be predictive of who might develop fatigue (see Table 3). Furthermore, there were no differences between fatiguers and nonfatiguers for any of the ventilatory or respiratory mechanical variables during exercise (data not shown). Nevertheless, the present study suggests that slightly more men develop fatigue and that the magnitude of fatigue appears to be greater in men. The findings from this study are consistent with the work of Gonzales and Scheuermann (11) who found a slower rate of inspiratory muscle fatigue during resistive breathing in women, despite using a less objective measure of fatigue (i.e., changes in maximal inspiratory pressure).

Absolute Load on the Diaphragm

The men and women in this study were exercising at the same relative intensity (90% of peak work rate), but the absolute work rate was higher in men. Therefore, it would be expected that the absolute load on the male respiratory system would be higher to accommodate their higher levels of absolute ventilation. Indeed, the total work of breathing was higher in men across the entire duration of the constant load cycling test (Fig. 7*E*), which is an observation consistent with recent work (14) that showed a higher work of breathing in men than in women at different percentages of maximal ventilation (i.e., a relative load). Women generally have less muscle mass than men, and this has been proposed as one of the key contributors to explain the greater fatigue resistance found in women for nonrespiratory skeletal muscles (23). Lower muscle mass may translate into lower absolute force generation in women when exercising at the same relative intensity as men. This lower absolute force production means there will be a decreased O_2 demand, a decrease in mechanical compression of the local vasculature, and less intramuscular occlusion of blood flow.

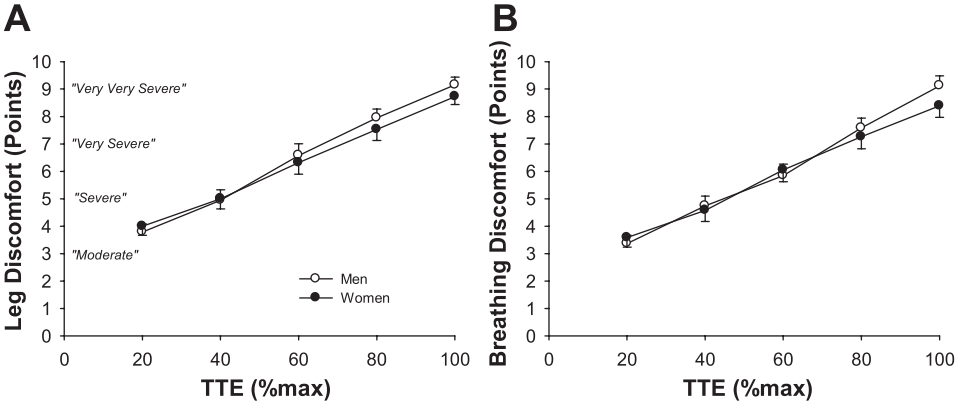

Fig. 6. Perception of leg (*A*) and breathing (*B*) discomfort in men and women. Values are mean ± SE. TTE, time to exhaustion. No differences in leg or breathing discomfort were detected at any time point between men and women.

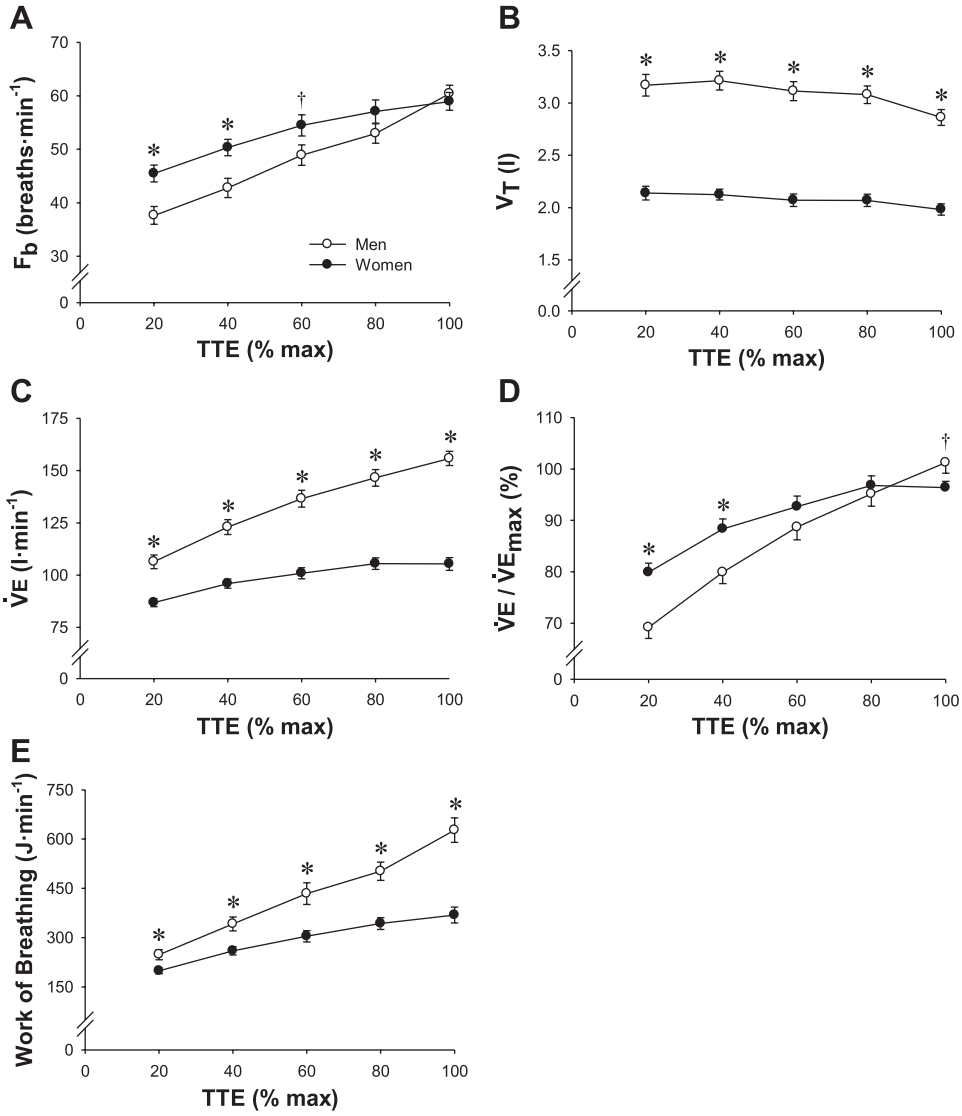

Fig. 7. Ventilatory (*A–D*) and work of breathing (*E*) responses to exercise in men and women. Values are means ± SE. Fb, breathing frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation; $\dot{V}E_{max}$, maximal minute ventilation from the incremental cycle test to exhaustion (*day 1*). Significant differences between men and women are shown (*$P < 0.01$; †$P < 0.05$).

The question relevant to the present study is whether we observed greater levels of diaphragmatic fatigue in men because they had higher absolute diaphragmatic force production during exercise. The men in this study did have higher absolute values for PTPdi during exercise, which may explain, in part, why men had greater levels of fatigue. The higher PTPdi over slightly longer exercise durations also means that the total load on the respiratory system was higher in men, which may have had a cumulative effect in causing more fatigue. In fact, the average PTPdi multiplied by exercise duration was ~34% higher in men, but this index of total load on the diaphragm did not correlate with diaphragm fatigue (data not shown). Although the higher load on the male diaphragm may partially explain our findings, there is some evidence to suggest that women still experience less skeletal muscle fatigue even when matched for both absolute force generation and maximal voluntary contraction (7). However, no study has looked at the diaphragmatic fatigue response in men and women matched for absolute diaphragmatic pressure production and matched subjects for maximal diaphragmatic strength. Other potential mechanisms to explain our findings include differences in muscular recruitment, muscle morphology, and substrate utilization (see below).

Ventilatory Response and Respiratory Muscle Recruitment

There are some important sex differences in the ventilatory response to exercise that may explain why men develop greater levels of diaphragmatic fatigue than women. Men have a progressive and linear increase in minute ventilation with increasing time spent at a fixed work rate (Fig. 7*C*). The male ventilatory response is nearly identical to what has been previously reported by Johnson et al. (26) in fit men exercising at 95% of their maximal O_2 consumption to exhaustion. The percent increase in minute ventilation from the early stages of exercise (20% TTE) to exhaustion (100% TTE) in our male subjects was 46%. This contrasts sharply to the modest increase in minute ventilation of 21% in women from 20% TTE to 100% TTE. In fact, the minute ventilation in women rises only 4% from 60% TTE to 100% TTE. Thus women have a plateau in minute ventilation despite performing the same muscular task; therefore, women use a smaller fraction of their

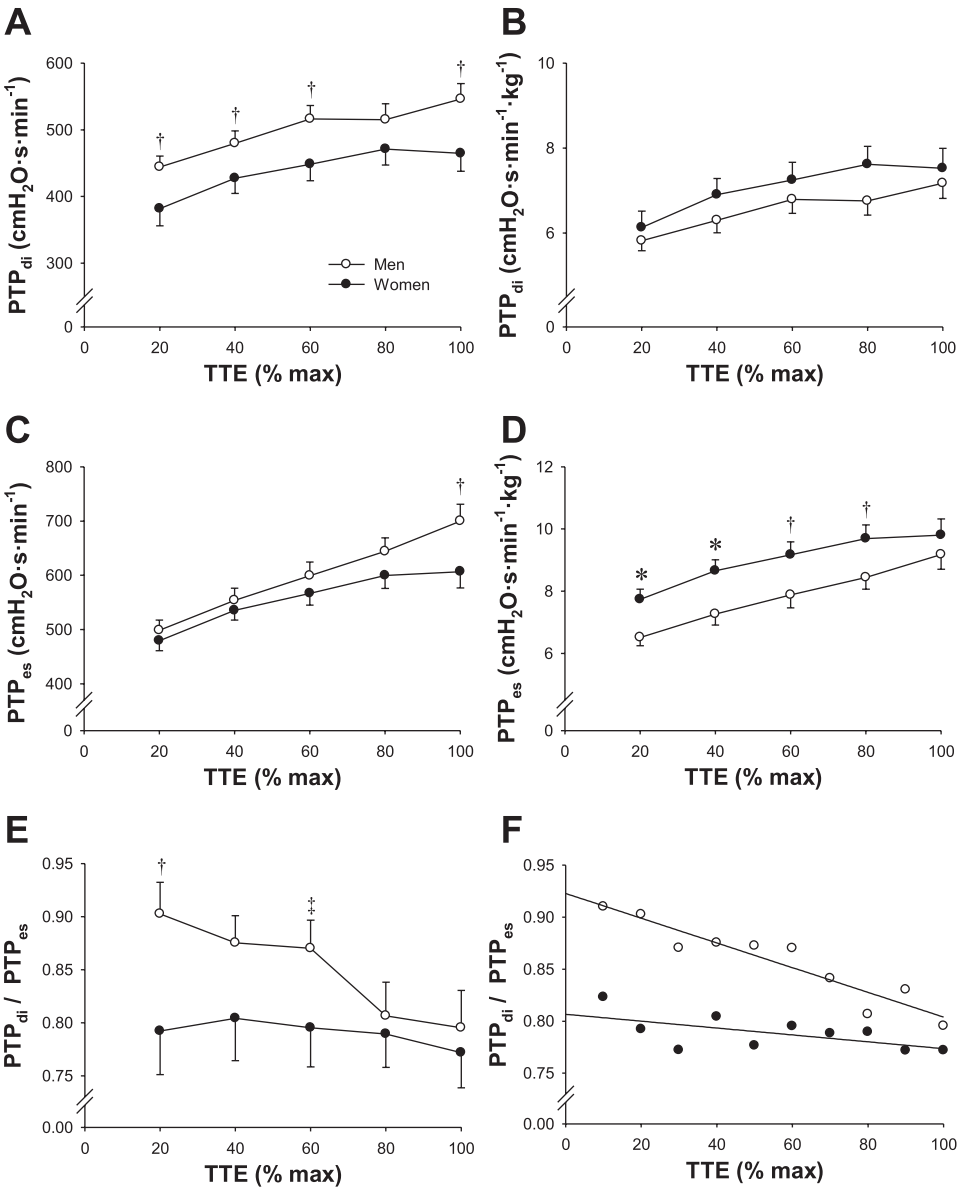

Fig. 8. *A–F*: diaphragmatic and esophageal pressure-time product responses (PTPdi and PTPes, respectively) to exercise in men and women. Values are means ± SE. Significant differences between men and women are shown (*$P < 0.01$; †$P < 0.05$; ‡$P = 0.05$).

maximal exercise ventilatory capacity at 100% TTE (Fig. 7*D*) than men. In fact, the minute ventilation-to-maximal ventilation ratio at 100% TTE was significantly, albeit modestly, related to the magnitude of diaphragmatic fatigue. It should be noted that we used minute ventilation-to-maximum ventilation ratio as an index of the physiological ventilatory capacity. True ventilatory capacity is measured as maximum voluntary ventilation, but we do not have these data, and the use of prediction equations to estimate the maximum voluntary ventilation is inappropriate in athletic populations. The higher ventilatory requirements in men during exercise might make the diaphragm more susceptible to fatigue and compromise its role as the primary inspiratory muscle. Why do men increase ventilation linearly over time, whereas women do not? There are several possible explanations for this. First, the women may have been mechanically constrained due to the potential presence of expiratory flow limitation. Although expiratory flow limitation was not assessed in the present study, our group (17) and others (33) have shown that women may be particularly susceptible to mechanical ventilatory constraints, which may be related to their smaller lungs and airways (45). However, this is unlikely to fully explain our findings since women appeared to have a slightly larger ventilatory reserve at 100% TTE than men. Alternatively, it is possible that the blunted ventilatory response in women during exercise was due to a reduced chemical drive to breathe. We have previously shown that chemosensitivity is not different between healthy men and women under resting conditions (13), but it is currently unknown whether there are sex differences in chemosensitivity during exercise. Finally, an inadequate hyperventilatory response is thought to be one of the mechanisms of exercise-induced arterial hypoxemia. However, the level of compensatory hyperventilation was probably similar between sexes, since we observed identical levels of arterial hypoxemia at exhaustion in our men and women.

What effect does this difference in the ventilatory response have on respiratory mechanics and muscle recruitment? Figure 8*A* demonstrates a continual rise in PTPdi across time for men,

whereas women plateau between 80% and 100% TTE. Figure 8*E* shows the relative contribution of the diaphragm to total inspiratory muscle force output, and Fig. 8*F* shows the slopes of these relationships for men and women. In relative terms, men rely less on the diaphragm over time, suggesting an increased recruitment of accessory inspiratory muscles to keep increasing minute ventilation. Women on the other hand show very little change in diaphragmatic contribution to total inspiratory force output, as shown by the slope in Fig. 8F. This is likely a function of their reduced ventilatory requirement. This change in recruitment pattern in men may be a physiological response to the onset of diaphragm fatigue, which persists well into recovery.

The potential role that PTPdi and respiratory muscle recruitment has on diaphragmatic fatigue remains difficult to determine. Although these potential sex differences in breathing mechanics are an attractive hypothesis to explain our findings, there are additional mechanisms of fatigue that must be considered. For example, recent work by Vogiatzis et al. (53) suggests that intercostal muscle blood flow increases linearly with the work of breathing during voluntary hyperpnea but decreases at the same work of breathing during whole body exercise at intensities above 80% of maximal work rate. Thus the circulatory system is unable to meet the demands of both locomotor and respiratory (intercostal) muscles during heavy exercise, which likely contributes to respiratory muscle fatigue. Although we have shown differences in diaphragmatic force production between sexes and differences in respiratory muscle recruitment, we recognize that there are other crucial factors such as blood flow competition that may explain our findings. Recent work has utilized near-infrared spectroscopy and a light-absorbing tracer (indocyanine green) to measure respiratory muscle blood flow during voluntary hyperpnea and exercise (16, 52, 53), but no such measurements have been made in women. Future work in this area is required to help explain the mechanisms underlying the greater fatigue resistance in women.

Muscle Morphology and Substrate Utilization

Some evidence suggests that there are sex differences in muscle fiber-type composition such that women have more slow-twitch oxidative fibers (36). Slow-twitch oxidative fibers fatigue at slower rates than fast-twitch glycolytic fibers (18). These potential sex differences in muscle fiber-type composition may explain, in part, why female muscles are more fatigue resistant than male muscles (23). In the present investigation, we are interested in the primary muscle of inspiration. The human diaphragm is composed of 76% high-oxidative fibers (55% slow twitch and 21% fast twitch) and 24% low-oxidative fast-twitch fibers (28). However, we are unaware of any studies that have looked specifically at sex differences in diaphragmatic fiber-type composition in healthy humans, particularly in endurance-trained individuals. There are also sex differences in substrate utilization (49), which may also contribute to potential sex differences in muscle fatigue. Hicks et al. (23) suggest that differences in substrate utilization may mean that women have a greater reliance on β-oxidation of fatty acids, thus prolonging endurance during certain types of exercise and perhaps improving their ability to resist fatigue.

Consequences of Diaphragm Fatigue

There are numerous studies pointing to a female respiratory system that may be more susceptible to specific pulmonary limitations, such as expiratory flow limitation (17, 33) and exercise-induced arterial hypoxemia (15, 21, 39). However, the data from the present study point to a female respiratory system that has a distinct advantage over the male respiratory system. That is, women are more resistant to exercise-induced diaphragmatic fatigue. There are several important physiological and performance-based consequences of diaphragmatic fatigue that have recently been reviewed by Romer and Polkey (40). We will briefly discuss some of these consequences in the context of the present data.

The greater reliance on accessory inspiratory muscles in men with progressive exercise may result in chest wall distortion (10, 12) and reduce the mechanical efficiency of breathing (22). The reliance and recruitment of accessory inspiratory muscles may lead to an increase in sensory input to the central nervous system, resulting in an increased sensation of breathlessness (40). However, despite the greater levels of fatigue in men, there appeared to be no sex differences in ratings of breathing or leg discomfort. Although respiratory muscle fatigue may increase the sensation of breathlessness (8, 48, 55), it is likely that this effect is specific to the accessory muscles because diaphragm fatigue does not increase neural respiratory drive as assessed by esophageal diaphragm EMG (29).

Another potential consequence related to diaphragm fatigue is a sympathetically mediated metaboreflex that originates from fatiguing inspiratory muscles. Fatigue-inducing inspiratory contractions cause a reduction in arterial blood flow to the resting limb (44). Harms et al. (20) found that reducing the inspiratory work of breathing using a mechanical ventilator causes vascular conductance and blood flow in the exercising limb to increase. Thus the sympathetically mediated vasoconstriction of locomotor limb muscle vasculature may lead to an exacerbation of peripheral fatigue, increase effort perceptions, and ultimately limit exercise performance (6). It is possible that men have a more pronounced inspiratory muscle metaboreflex response given that they tend to exhibit greater levels of diaphragm fatigue. A sex-based comparison of the inspiratory metaboreflex is required to fully address this hypothesis.

Methodological Considerations

Supramaximal stimulation of the phrenic nerves can be confirmed by showing a plateau in Pdi,tw with increasing power output of the stimulator. Figure 2 shows that, on average, men and women have a leveling off in Pdi,tw at 90–95% of stimulator output. However, fewer men reached a plateau in Pdi,tw than women, likely because the men were generally taller with thicker necks. However, we do not believe that submaximal stimulation in some of our men would influence our primary finding of greater fatigue resistance in women for several reasons. First, Verges et al. (51) found that only 4 of 11 subjects showed evidence of a plateau when comparing Pdi,tw at 94% of the stimulator output to 100%; however, a plateau was seen in 8 of 11 subjects when comparing Pdi,tw at 98% of the stimulator output to 100%. We made our measurements at larger increments on the stimulator output than Verges et al. (51). Our more conservative approach probably led to an underestimation of the number of subjects showing a plateau in

Pdi,tw, and thus suggesting that stimulations may have been submaximal. Had we performed our stimulations at 98% of the stimulator power output, we believe we would have demonstrated a plateau in most of our male subjects. Second, M-wave amplitude was unchanged after exercise in both men and women, suggesting that the reduction in Pdi,tw was not due to derecruitment of muscle fibers or to transmission failure. Third, the stimulator output was set at 100% of maximal power output for all stimulations, and we paid particular attention to ensure that the subjects were in the same position before and after exercise. In addition to visual inspection of the subject while seated, we also marked the coil position on the back of the neck, which allowed us to reposition the coil in the exact location for all stimulations. The consistency of our stimulations before and after exercise can be seen by the lack of change in the coefficient of variation in Pdi,tw. Finally, any twitches that were not initiated from resting EELV were discarded from the analysis. Therefore, although supramaximal stimulations were not obtained in all men, we are confident that our stimulations were constant and that this limitation would not influence our primary finding regarding sex differences in diaphragmatic fatigue.

In the present study we did not confine our testing to a certain phase of the menstrual cycle. This was done because we wanted our findings to have greater practical implications for exercising women. Confining our testing to one specific phase would have limited our interpretation to a very brief period of time in a woman's cycle. Testing women randomly throughout the menstrual cycle eliminates the potential influence that reproductive hormone concentrations might have on our results. There is some evidence to suggest that skeletal muscle (quadriceps) fatigue is not affected by fluctuations in hormone concentrations throughout the menstrual cycle (25). However, it is currently unknown whether a similar finding can be extended to the respiratory muscles. Although controversial, previous work has suggested that respiratory function may be influenced by female sex hormones (41, 57). Thus we cannot rule out the possibility that our results may have been different if we standardized to specific phases of the menstrual cycle. Future work is needed in women to determine whether exercise-induced diaphragmatic fatigue is affected by hormonal fluctuations.

The subjects used in this study were well-trained endurance athletes with a high aerobic capacity. We used athletes because they are highly motivated and are able to stress their cardiorespiratory system well beyond their untrained or diseased counterparts. Thus athletes provide an excellent model to study the limitations of the human respiratory system during exercise. However, endurance athletes represent a small fraction of the general population, and thus the generalizability of our findings are limited to the population from which we derived our data. For example, how our findings apply to other populations such as untrained individuals, healthy ageing, obesity, and those with chronic diseases remains unknown and requires further investigation.

In conclusion, this study is the first to measure diaphragm function during exercise in healthy trained women and the first to use objective measures to investigate diaphragm fatigue in a large group of women. The data from the present study point to a diaphragm that is more resistant to exercise-induced fatigue in women than in men. We have also shown sex differences in the ventilatory response to high-intensity constant-load cycling exercise that may be related, in part, to the greater fatigue resistance observed in women.

ACKNOWLEDGMENTS

We are indebted to our research participants for patience, commitment, and enthusiastic participation in this study. We also thank Drs. Ian M. Franks, J. Timothy Inglis, and David J. Sanderson from the School of Human Kinetics at the University of British Columbia and Dr. David Goodman from the Department of Biomedical Physiology and Kinesiology at Simon Fraser University for allowing us to use their Magstim 200 unit, which was purchased from an equipment grant funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

GRANTS

This study was supported by the NSERC and the British Columbia Lung Association. J. A. Guenette was supported by graduate scholarships from NSERC, the Michael Smith Foundation for Health Research (MSFHR), and the Sir James Lougheed Award of Distinction. J. S. Querido was supported by graduate scholarships from the MSFHR and the Heart and Stroke Foundation of Canada. A. W. Sheel was supported by a Scholar Award from the MSFHR and a New Investigator award from the Canadian Institutes of Health Research.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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