EXERCISE-INDUCED DIAPHRAGMATIC FATIGUE IN HEALTHY HUMANS

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SUMMARY

1. Twelve healthy subjects (33 ± 3 years) with a variety of fitness levels (maximal oxygen uptake \( \dot{V}_{O_2, \text{max}} \) = 61 ± 4 ml kg\(^{-1}\) min\(^{-1}\), range 40–80), exercised at 95 and 85% \( \dot{V}_{O_2, \text{max}} \) to exhaustion (mean time = 14 ± 3 and 31 ± 8 min, expired ventilation \( \dot{V}_E \) over final minute of exercise = 149 ± 9 and 126 ± 10 l min\(^{-1}\)).

2. Bilateral transcutaneous supramaximal phrenic nerve stimulation (BPNS) was performed before and immediately after exercise at four lung volumes, and 400 ms tetanic stimulations were performed at 10 and 20 Hz. The coefficients of variation of repeated measurements for the twitch transdiaphragm pressures \( P_{\text{di}} \) were ±7–10% and for compound muscle action potentials (M wave) ±10–15%.

3. Following exercise at 95% of \( \dot{V}_{O_2, \text{max}} \), group mean \( P_{\text{di}} \) twitch values were reduced at all lung volumes (range –8 ± 3 to –32 ± 5%) and tetanically stimulated \( P_{\text{di}} \) values were reduced at both 10 and 20 Hz (–21 ± 3 and –13 ± 2%, respectively) \( (P = 0.001–0.047) \). Following exercise at 85% \( \dot{V}_{O_2, \text{max}} \), stimulated \( P_{\text{di}} \) values were reduced at all lung volumes and stimulating frequencies, but only significantly so with the twitch at functional residual capacity (–15 ± 5%). Stimulated \( P_{\text{di}} \) values recovered partially by 30 min post-exercise and almost completely by an average time of 70 min.

4. The fall in stimulated \( P_{\text{di}} \) values post-exercise was significantly correlated with the percentage increase in diaphragmatic work \( (\int P_{\text{di}} \text{ min}^{-1}) \) from rest to end-exercise and the relative intensity of the exercise.

5. The \( \int P_{\text{di}} \text{ min}^{-1} \) and the \( \int P_o \text{ min}^{-1} \) (\( P_o \), oesophageal pressure) rose together from rest through the fifth to tenth minute of exercise, after which \( \int P_{\text{di}} \text{ min}^{-1} \) plateaued even though \( \int P_o \text{ min}^{-1} \), \( \dot{V}_E \) and inspiratory flow rate all continued to rise substantially until exercise terminated. Thus, the relative contribution of the diaphragm to total respiratory motor output was progressively reduced with exercise duration.

6. We conclude that significant diaphragmatic fatigue is caused by the ventilatory requirements imposed by heavy endurance exercise in healthy persons with a variety of fitness levels. The magnitude of the fatigue and the likelihood of its occurrence increases as the relative intensity of the exercise exceeds 85% of \( \dot{V}_{O_2, \text{max}} \).
INTRODUCTION

Numerous studies have attempted to determine if the respiratory muscles, particularly the diaphragm, fatigue during whole body exercise and if so, what the implications may be for exercise performance (Shephard, 1967; Loke, Mahler & Virgulto, 1982; Martin, Heintzelman & Chen, 1982; Bye, Esau, Walley, Macklem & Pardy, 1984; Fregosi & Dempsey, 1986; Levine & Henson, 1988; Gallagher & Younes, 1989; Coast, Clifford, Henrich, Stray-Gunderson & Johnson, 1990). The issue remains controversial mainly due to the indirect techniques used to assess respiratory muscle fatigue. Previous studies in humans have focused on the use of volitional tests of maximum mouth, pleural or transdiaphragm pressure development (Loke et al. 1982; Bye et al. 1984; Hussain & Pardy, 1985; Coast et al. 1990), shifts in the frequency spectrum of the diaphragm integrated electromyographic activity (EMG) (Bye et al. 1984), unloading the respiratory muscles (Aaron, Henke, Pegelow & Dempsey, 1985; Gallagher & Younes, 1989) or by mimicking at rest the ventilation achieved during exercise for given time periods (Aaron, Seow, Johnson & Dempsey, 1992; Shepherd, 1967). The results of the volitional tests are inconclusive because the test conditions themselves are difficult to control and are not sufficiently objective or independent of total body fatigue. The validity of using an index of fatigue such as the spectral changes in the EMG frequency has also been challenged (Sieck & Fournier, 1990).

The technique of supramaximal bilateral phrenic nerve stimulation (BPNS), has shown that the normal human diaphragm is fatigable during prolonged bouts of loaded breathing or during isocapnic hyperpnoea performed at rest (Bellemare & Bigland-Ritchie, 1984; Bai, Rabinovitch & Pardy, 1984; McKenzie & Gandevia, 1991). The nerve stimulation technique has also been applied to conditions of short term progressive exercise to exhaustion, following which diaphragmatic fatigue was not observed unless external resistive loads were added during the exercise (Levine & Henson, 1988).

The purpose of our study was to use the BPNS technique to determine the effects of endurance exercise to exhaustion on diaphragmatic function in normal, healthy subjects with a broad range of fitness levels from the sedentary to the highly trained. In addition, we (a) quantified the relative contribution of the diaphragm to total inspiratory muscle pressure development throughout the time course of endurance exercise; and (b) determined the degree to which the diaphragm used its available capacity for pressure development during exercise.

METHODS

Subjects

We studied twelve male subjects with a mean age of 33 ± 3 years (mean ± S.E.M.) who had resting lung volume subdivisions and maximal flow rates which were within ±10% of normal predicted values. Subjects were chosen to represent a range of fitness levels (i.e. mean maximal \( V_{O_2, \text{max}} \) = 61 ± 4, range 40–80 ml kg\(^{-1}\) min\(^{-1}\) and maximal exercise ventilation of 157 ± 7, range 122–193 l min\(^{-1}\)).

Bilateral phrenic nerve stimulation

Transcutaneous BPNS was performed following the procedure of Bellemare & Bigland-Ritchie (1984). Stimulating electrodes were adjusted to deliver pulses of 100 μs duration with a current that ranged between 0 and 80 mA. The stimulator (Tracor, model 3600) was a constant current
stimulator with an arming voltage of up to 400 V. Stimulation frequencies during testing were 1, 10 and 20 Hz. During the 10 and 20 Hz stimulation, the train duration lasted approximately 350–400 ms.

To ensure that the phrenic nerves were supramaximally stimulated, a pair of EMG surface electrodes were placed over each hemidiaphragm in the sixth or seventh intercostal space midway between the anterior axillary and midaxillary line on each side of the thorax. In addition, to make sure that there were no conduction changes with the surface electrodes due to sweating, some subjects inserted a polyethylene tube (PE 200) intranasally with multiple EMG electrodes positioned at the gastro-oesophageal junction and secured with a small balloon. The EMG electrodes were attached to a preamplifier (Tracor, model 3600) and connected to the Tracor computer as well as to a Gould recorder (model ES2000) for further amplification, filtering and analysis. When the phrenic nerves were stimulated, diaphragmatic mass action potentials (i.e. M waves) were recorded from each hemidiaphragm.

For pressure measurements, subjects passed oesophageal and gastric balloons via the nose. Our use of balloons for measurement of oesophageal ($P_o$), gastric ($P_g$) and transdiaphragm ($P_{di}$) pressure has been previously described (Henke, Sharratt, Pegelow & Dempsey, 1988).

To locate the phrenic nerves, we stimulated once per second at a submaximal current until the largest M wave was found. We then increased current until the M wave amplitude reached a plateau. We also monitored $P_{di}$ which reached a plateau in amplitude in parallel with the M wave. The position was then marked on the neck for future reference. During test stimulations, the current was increased 50% above that needed to obtain maximal M wave amplitude to assure supramaximal stimulation. This procedure for obtaining the maximum M wave was repeated for each test session (i.e. pre- and post-exercise).

The BPNS (1 Hz ‘twitch’) was performed at total lung capacity (TLC), half-inspiratory capacity (IC), functional residual capacity (FRC) and residual volume (RV) while subjects were relaxed and the airway was occluded. Stimulations were repeated until 8–10 reproducible measurements for $P_{di}$ and M wave amplitude were obtained at FRC and 3–5 such measurements at the other lung volumes. Lung volumes were continuously monitored throughout the tests by connecting subjects to a wedge spirometer and providing feedback on lung volume to the subject by an oscilloscope display.

The BPNS at 10 and 20 Hz was always performed at each subject’s FRC. Both the twitches and the 10 and 20 Hz stimulation at FRC were accepted for measurement only if the subjects were within $\pm 10\%$ of this resting lung volume.

To minimize the possibility of volitional effort during tetanic stimulation, pressure ($P_o$ and $P_g$) signals were monitored continuously to ensure that subjects were in a relaxed state prior to stimulation. Furthermore, stimulation time was kept to less than 400 ms and following stimulation, $P_o$ and $P_g$ were observed to return simultaneously to prestimulus control values.

The M waves were stored and monitored after each twitch or series of pulses. A given stimulation was repeated if the M wave amplitude was reduced by $> 10-15\%$ compared to the established maximal M wave for that phase of study (i.e. pre- or post-exercise) or if the $P_{di}$ baseline shifted significantly from that measured at the end of an expiration during quiet breathing.

During all the stimulation procedures pre- or post-exercise, subjects were fixed in a semi-recumbent position.

**Measurement of diaphragmatic twitches**

Our $P_{di}$ twitches were analysed according to the following definitions. Peak tension was defined as the maximum increase in twitch tension above the $P_{di}$ baseline. Contraction time (CT) represented the time interval between the initiation of twitch tension and peak tension. Relaxation time (RT) was the time necessary for $P_{di}$ to decrease to one-half of the peak tension. Relaxation time constant (RTC) was determined by (a) performing a log transformation of points taken from the downward slope of the twitch $P_{di}$ waveforms and (b) determining the slope of the linear regression line plotted through these points (Esau, Bye & Pardy, 1983).

**Volitional measurements of diaphragm muscle force**

Just prior to each exercise session and immediately post-exercise, tests were performed to assess maximum volitional $P_{di}$. These tests consisted of two to three maximal inspiratory manoeuvres against an occluded airway (Mueller manoeuvres), and maximal inspiratory manoeuvres combined with an expulsive manoeuvre (Laporta & Grassino, 1985). All tests were performed at FRC with feedback of $P_e$ and $P_r$ provided for the subject on an oscilloscope.
Measurements of mechanical constraint

The open-circuit system for breath-by-breath measurement of inspiratory and expiratory flow rates, volumes, pressures, end-tidal $P_{O_2}$, $P_{CO_2}$ and end-expiratory lung volume (EELV) during exercise has been described in detail (Henke et al. 1988; Johnson, Seow, Pegelow & Dempsey, 1990).

To determine how close subjects came to reaching expiratory flow limitation, tidal exercise flow:volume loops were measured, averaged over twenty to thirty breaths, and plotted within a maximal volitional flow:volume envelope (MFVL) using a measured EELV (Johnson, Reddan, Seow & Dempsey, 1991). Prior to exercise we also measured the maximal effective pressure generation ($P_{max,e}$) throughout expiration over a range of lung volumes, to determine if and when tidal expiratory pressures during exercise exceeded their effective limit for flow generation (Olafsson & Hyatt, 1969; Johnson et al. 1991).

The capacity ($P_{cap,i}$) of all the inspiratory muscles to generate $P_a$ at different flow rates (velocity of shortening) and at various lung volumes (muscle lengths) was determined at rest using techniques similar to those previously described and a regression equation computed for each subject (Johnson, Saupe & Dempsey, 1992). We then identified the peak inspiratory $P_a$ reached during tidal inspiration in exercise and applied the corresponding volume and flow rate to the multiple regression equation to determine the available capacity for pressure generation ($P_{cap,i}$). The difference in peak $P_a$ between the tidal inspiration and the $P_{cap,i}$ represented the demand capacity margin for inspiratory pressure development during exercise.

We used a similar approach to estimate the available capacity for transdiaphragm pressure generation during exercise ($P_{cap,di}$). We determined the maximal volitional combined expulsive and Mueller manoeuvre (for $P_{di}$) with the airway occluded at FRC for each subject and used the regression equation obtained for $P_{a}$ (see above) to subtract out the effects of lung volume and flow rate on the ability to generate $P_{di}$. These derived maximal available $P_{di}$ values for a given lung volume and flow rate agreed fairly closely with those obtained during maximum volitional dynamic manoeuvres such as the maximal voluntary ventilation (MVV) and the MFVL. Just as with $P_{cap,i}$ (see above), $P_{cap,di}$ represented an index of the maximum pressure ‘available’ to the diaphragm for any given flow rate and lung volume achieved during exercise hyperpnoea.

Exercise test protocols

The first exercise test determined the subject’s $V_{O_2,max}$ using a progressive, short-term test to volitional exhaustion, as previously described (Johnson et al. 1992). Ten of the subjects ran on a treadmill and two exercised on a stationary bicycle.

Each endurance exercise session was conducted on a separate test day using the following protocol. First the various measurements using BPNS were obtained. Subjects then warmed up briefly with light exercise and were then quickly brought up to the work intensity which elicited 90–95% or 80–85% of their $V_{O_2,max}$ which they maintained until volitional exhaustion. Immediately post-exercise, an identical protocol was followed for BPNS and volitional tests as were used pre-exercise. The BPNS protocol was completed by 10–15 min post-exercise and then repeated every 20–30 min until pressure measurements were back to baseline.

End-expiratory lung volume and IC measurements were repeated at 2–3 min intervals and expired gases, flow, volume, $P_a$, mouth pressure ($P_m$), $P_e$ and $P_{di}$ were monitored continuously throughout exercise. For analysis, data from twenty to thirty consecutive breaths were averaged at 2–3 min intervals throughout exercise.

Statistical analyses were performed with the statistical program Systat. Significant differences between mean values were determined by ANOVA for repeat measurements. Correlation coefficients were the Pearson product moment correlations. Coefficients of variation for repeated measures obtained with BPNS were determined by the s.d. differences/grand mean multiplied by 100.

RESULTS

Exercise response

The ventilatory response to exercise at 95 and 85% of $V_{O_2,max}$ is shown in Fig. 1 and the group mean flow–volume, and pleural pressure–volume response shown in Fig. 2. Subjects averaged to 96±3% and 88±5% of their $V_{O_2,max}$ over the last 2 min of each respective exercise intensity. Exercise time averaged 14±3 min (range 9–18)
at 95% of $\bar{V}_{O_2,\text{max}}$ and 31 ± 8 min (range 23–38) at 85% of $\bar{V}_{O_2,\text{max}}$. From the third to the final minute of the two exercise intensities (95 and 85% respectively), rate of O₂ uptake ($\bar{V}_{O_2}$) rose 19 ± 3 and 20 ± 5% and rate of CO₂ expiration ($\bar{V}_{CO_2}$) increased 23 ± 5% and 21 ± 5%. Expiratory ventilation rate ($\bar{V}_E$) rose an average of 46 ± 7% throughout the 95% work load and 41 ± 9% throughout the 85% work load, due exclusively to an increased breathing frequency at a constant tidal volume ($V_T$). $\bar{V}_E$: $\bar{V}_{CO_2}$ rose 18% throughout both exercise loads. End-expiratory lung volume fell approximately 0.5 l within the first few minutes of both exercise work rates and subsequently rose 0.2 l by the final minutes of exercise.

Expiratory flow limitation began to occur at the mid-time point of each exercise load and continued to increase over exercise time so that over the final minutes of exercise, flow limitation was present over 42 ± 8% of the $V_T$ at the 95% work load and 28 ± 9% at the 85% level. During the final few minutes at the higher work intensity, the capacity for $P_o$ development during inspiration ($P_{\text{cap},i}$) fell 59 cmH₂O or 60% from rest, due to the high lung volume at which peak-tidal inspiratory $P_o$ occurred (75–80% of TLC) and the high peak-tidal inspiratory flow rates (5.7 ± 0.4 l s⁻¹) achieved during exercise. At the same time, the negative swing in peak $P_o$ also achieved during tidal breathing increased to −31 ± 2 cmH₂O; thus during the final minutes of exercise at 95% of $\bar{V}_{O_2,\text{max}}$, 67 ± 5% of the available $P_{\text{cap},i}$ was used (range 44–100%). Comparable values at the termination of the 85% work load were a 52% reduction in $P_{\text{cap},i}$ and a tidal pleural pressure of −27 ± 2 cmH₂O; thus, 58 ± 6% of $P_{\text{cap},i}$ was achieved during tidal breathing.
The top two panels of Fig. 3 show the average time integrals for $P_o$ and $P_{di}$ ($\int P_o \, \text{min}^{-1}$, $\int P_{di} \, \text{min}^{-1}$) and the ratio of the $\int P_{di} \, \text{min}^{-1}$ relative to $\int P_o \, \text{min}^{-1}$ throughout each exercise session. During exercise at the 95% work rate, the $\int P_o \, \text{min}^{-1}$ continued to rise steadily throughout exercise, increasing an average of $44 \pm 9\%$ from minute three to minute fourteen; whereas the $\int P_{di} \, \text{min}^{-1}$ rose only $16 \pm 7\%$ within the same time period. Similarly during the 85% work rate, the $\int P_{di} \, \text{min}^{-1}$ remained constant during exercise while the $\int P_o \, \text{min}^{-1}$ rose an average of $20 \pm 9\%$ over exercise time. Thus, the ratio of the $\int P_{di} : \int P_o$ fell $14 \pm 7\%$ and $12 \pm 11\%$ throughout the exercise periods at 95 and 85% of $\dot{V}_{O_2,\text{max}}$ respectively.

Also shown in the bottom panel of Fig. 3 is peak $P_{di}$ relative to its estimated
EXERCISE-INDUCED DIAPHRAGMATIC FATIGUE

Fig. 3. Top (95% work rate) and middle (85% work rate) panels show $fP_{o_{2}}$ min$^{-1}$ (○) and $fP_{di}$ min$^{-1}$ (●) during inspiration multiplied by the breathing frequency, as an index of inspiratory muscle work min$^{-1}$ vs. exercise time. Also shown in these two panels is the ratio of the $fP_{di}$ min$^{-1}$ relative to the $fP_{o_{2}}$ min$^{-1}$ (*) throughout each of the exercise work rates. The bottom panel shows peak $P_{di}$ achieved during tidal breathing relative to the available $P_{di}$ for the flow rate and lung volume achieved at peak pressure during exercise (95% of $V_{O_{2-max}}$, ●; 85% of $V_{O_{2-max}}$, ○).

capacity ($P_{cap,di}$) during exercise. Peak $P_{di}$ plateaued during both exercise intensities, however, the $P_{cap,di}$ continued to fall over exercise time primarily because of an increasing flow rate. Thus, during the last few minutes of exercise at both work rates,
Table 1. Reproducibility of twitch pressures and M wave amplitudes at FRC within a given test session

<table>
<thead>
<tr>
<th>No. of twitches</th>
<th>Pdi</th>
<th>RMW</th>
<th>LMW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>95%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10·4</td>
<td>9·4</td>
<td>7·4</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0·6</td>
<td>0·7</td>
<td>0·8</td>
</tr>
<tr>
<td>85%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8·4</td>
<td>7·3</td>
<td>9·7</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0·5</td>
<td>0·4</td>
<td>1·1</td>
</tr>
</tbody>
</table>

Pre, values from BPNS performed prior to exercise. Post, values from BPNS obtained immediately following exercise. Coefficient of variation (s.D./mean value) x 100. RMW and LMW, right and left side diaphragmatic M waves.

Table 2. Reproducibility of M wave peak amplitude pre- vs. post-exercise at different lung volumes and stimulating frequencies

<table>
<thead>
<tr>
<th></th>
<th>RMW</th>
<th>LMW</th>
<th>RMW</th>
<th>LMW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean amplitude*</td>
<td>Mean amplitude</td>
<td>Mean amplitude</td>
<td>Mean amplitude</td>
</tr>
<tr>
<td></td>
<td>¤Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>TLC</td>
<td>49·6</td>
<td>62·4</td>
<td>62·0</td>
<td>55·9</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>7·0</td>
<td>11·0</td>
<td>7·4</td>
<td>9·1</td>
</tr>
<tr>
<td>Half</td>
<td>63·6</td>
<td>57·9</td>
<td>55·1</td>
<td>50·4</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>6·9</td>
<td>6·3</td>
<td>4·8</td>
<td>6·0</td>
</tr>
<tr>
<td>FRC</td>
<td>54·4</td>
<td>50·6</td>
<td>46·5</td>
<td>51·2</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>4·3</td>
<td>4·1</td>
<td>3·2</td>
<td>5·1</td>
</tr>
<tr>
<td>RV</td>
<td>45·2</td>
<td>42·4</td>
<td>45·9</td>
<td>47·2</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>5·6</td>
<td>5·6</td>
<td>4·1</td>
<td>4·7</td>
</tr>
<tr>
<td>10 Hz</td>
<td>48·1</td>
<td>52·0</td>
<td>48·2</td>
<td>52·3</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>4·7</td>
<td>7·8</td>
<td>4·7</td>
<td>5·1</td>
</tr>
<tr>
<td>20 Hz</td>
<td>45·7</td>
<td>52·4</td>
<td>45·5</td>
<td>55·1‡</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>4·8</td>
<td>6·7</td>
<td>5·0</td>
<td>4·3</td>
</tr>
</tbody>
</table>

Results given as means±S.E.M. Half, twitches performed at half-inspiratory capacity.

* M wave peak amplitude determined in arbitrary units.

† Pre, values obtained prior to exercise; Post, values obtained immediately after exercise.

‡ Post-exercise values significantly different from pre-exercise values, $P < 0·05$.

62±5% of the available capacity for pressure generation by the diaphragm was used.

Reproducibility of stimulated pressures and M wave amplitude:

The reproducibility of twitch pressures performed repeatedly at FRC within a given test session (i.e. before or after exercise) are shown in Table 1. For seven to fifteen twitches performed on each subject, the coefficient of variation averaged ±7–10% for $P_{di}$ and ±10–15% for M wave amplitude. Table 2 shows M wave amplitude pre- vs. post-exercise. No significant differences were observed except for
two of the test conditions where the M wave amplitude post-exercise was increased relative to pre-exercise. Mean lung volume subdivisions measured just prior to BPNS were also unchanged, pre- vs. post-exercise, for all the conditions tested ($P > 0.10$).

**Diaphragmatic response to electrical stimulation**

Figure 4 shows the mean peak twitch values for $P_{di}$ in response to BPNS at different lung volumes before and at $10 \pm 2$ min after exercise at 95% of $\dot{V}_{O_2, \text{max}}$. There were significant declines in the amplitude of the twitch $P_{di}$ values at each lung volume post-exercise ($P < 0.05$). The fall in pressure averaged between $9 \pm 2\%$ and $33 \pm 8\%$ progressing from RV to TLC. Table 3 shows group mean twitch responses to BPNS at FRC. Nine of the eleven subjects showed significant falls in $P_{di}$ (range 4–27%), contributed to by a reduction in $P_g$ and more so in $P_o$. The $P_{di}$ waveform RTC also was significantly prolonged post-exercise relative to pre-exercise values.
Similar findings to those obtained with supramaximal twitches post-exercise (95% \( \dot{V}_{O_2, \text{max}} \)) were also found with stimulation at 10 and 20 Hz (Fig. 4). The decreases averaged 13 \( \pm \) 2% with 20 Hz stimulation (\( P_o \) decreased 15% and \( P_g \) decreased 10%) and 21 \( \pm \) 3% with 10 Hz stimulation (\( P_o \) decreased 22% and \( P_g \) decreased 18%). The third panel of Fig. 4 shows an identity plot for \( P_{di} \) stimulation pre- and post-exercise at 95% of \( V_{O_2, \text{max}} \) for all subjects. As shown by the regression line, the majority of points fell significantly below the line of identity.

Figure 5 shows a typical example of the raw data obtained on a subject who showed significant reductions in \( P_{di} \) following exercise at the 95% work rate when stimulated at FRC with 1, 10 and 20 Hz.

Table 4 and Fig. 6 show the response to BPNS before and after exercise at the 85% work rate. Mean \( P_{di} \) values fell post-exercise at all lung volumes with supramaximal twitches and at 10 and 20 Hz. These effects following exercise at 85% of \( \dot{V}_{O_2, \text{max}} \) were not as great or as consistent as those following exercise at 95% \( \dot{V}_{O_2, \text{max}} \) and only reached significance (\( P < 0.05 \)) with the supramaximal twitch at FRC and with 10 Hz stimulation where four of the eight subjects demonstrated significant decreases. No other indices of fatigue including RTC were significantly different from pre-exercise values.

**Volitional tests**

Pre- and post-exercise volitional manoeuvres are shown in Table 5. Post-exercise \( P_{di} \), obtained by the Mueller manoeuvre, was not significantly different from pre-exercise following either exercise intensity. However, we did find significant differences in the \( P_{di} \) post-exercise obtained by the combined Mueller and expulsive manoeuvre for both exercise conditions. We note however, that many subjects had difficulty in performing the latter manoeuvre immediately post-exhaustive exercise despite the use of visual feedback of the pressure signals.
Fig. 5. Individual examples of BPNS in a subject who demonstrated significant fatigue post-exercise at 95% of $V_{O_2,max}$. The figure shows the $P_o$, $P_g$, and $P_{di}$ and the M waves from the left (LD) and right (RD) diaphragm. All stimulations were performed at FRC: top panel, 1 Hz; middle panel, 10 Hz; and bottom panel, 20 Hz. Note that the pressure measurements were increased two-fold in sensitivity at 20 Hz stimulation vs. those at 1 and 10 Hz.
Fig. 6. Response to BPNS before (●) and after (○) exercise at 85% of $\dot{V}_{O_2,\text{max}}$. Left panel shows the response to supramaximal twitches delivered at different lung volumes; right panel shows the response to stimulation at FRC with 1, 10 and 20 Hz stimulation. Bottom panel is an identity plot showing the identity line (continuous) and regression line (dashed) for phrenic nerve stimulation at 1, 10 and 20 Hz in all subjects (* post-exercise mean values significantly different from pre-exercise, $P < 0.05$).

Table 4. Group mean twitch parameters at FRC, pre- vs. post-exercise ($n = 8$) at 85% of $\dot{V}_{O_2,\text{max}}$

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.e.m.</td>
<td>Mean ± s.e.m.</td>
</tr>
<tr>
<td>$P_{di}$ (cmH$_2$O)</td>
<td>27.5 ± 2.0</td>
<td>*22.6 ± 2.8</td>
</tr>
<tr>
<td>$P_e$ (cmH$_2$O)</td>
<td>12.8 ± 1.2</td>
<td>*10.9 ± 1.7</td>
</tr>
<tr>
<td>$P_o$ (cmH$_2$O)</td>
<td>14.8 ± 1.1</td>
<td>*12.2 ± 1.3</td>
</tr>
<tr>
<td>CT (s)</td>
<td>0.10 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>RT (s)</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>RMW (au)</td>
<td>62.7 ± 9.4</td>
<td>59.3 ± 11.4</td>
</tr>
<tr>
<td>LMW (au)</td>
<td>53.5 ± 7.9</td>
<td>49.4 ± 6.3</td>
</tr>
<tr>
<td>Lung volume (% TLC)</td>
<td>53.5 ± 24</td>
<td>53.4 ± 27</td>
</tr>
<tr>
<td>RTC (ms)</td>
<td>62.9 ± 5.2</td>
<td>60.4 ± 4.5</td>
</tr>
</tbody>
</table>

* Significant difference between pre- and post-exercise for group mean values ($P < 0.05$).

CT, contraction time; RT, half-relaxation time; RMW and LMW, right and left diaphragm M waves in arbitrary units (au); RTC, relaxation time constant of the twitch $P_{di}$ waveform (see Methods).
EXERCISE-INDUCED DIAPHRAGMATIC FATIGUE

Fig. 7. Time course of recovery after exercise at 95% of $\dot{V}_{O_{2max}}$. Left panel shows pre-exercise twitch response at different lung volumes (time 0) and the subsequent times of stimulation post-exercise. The right panel shows the response to 10 and 20 Hz stimulation pre-exercise (time 0) and post-exercise (means ± S.E.M.). * Significant differences between pre- and post-exercise determined at $P < 0.05$. HLF refers to measurements made during 1 Hz phrenic stimulation at half-inspiratory capacity.

Table 5. Volitional manoeuvres performed before and after exercise

<table>
<thead>
<tr>
<th></th>
<th>95% Exercise</th>
<th>85% Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mueller</td>
<td>Expulsive + Mueller</td>
</tr>
<tr>
<td>$P_{di}$ (cmH₂O)</td>
<td>Pre Post 108-9 108-2 *167-4</td>
<td>97-1</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>8-1 7-2 6-1 7-3</td>
<td>7-1 5-6</td>
</tr>
<tr>
<td>$P_s$ (cmH₂O)</td>
<td>Pre Post 18-9 12-0</td>
<td>15-6 3-3</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>8-4 7-7 7-2 6-8</td>
<td>7-1 5-6</td>
</tr>
<tr>
<td>$P_a$ (cmH₂O)</td>
<td>Pre Post 90-4 89-6</td>
<td>92-8 93-8</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>4-9 3-8 5-9 3-7</td>
<td>4-0 4-3</td>
</tr>
<tr>
<td>Lung volume (% TLC)</td>
<td>Pre Post 50-8 52-5</td>
<td>51-9 51-1</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>1-7 2-1 2-0 2-0</td>
<td>1-8 1-5</td>
</tr>
</tbody>
</table>

Results are given as means ± S.E.M.

* Significant difference between pre- and post-exercise at $P < 0.05$.

Post-exercise recovery

The time course of recovery with BPNS in seven subjects after exercise at 95% of $\dot{V}_{O_{2max}}$ is shown in Fig. 7. As shown, there was a trend toward recovery in every condition except for 20 Hz stimulation, by the average time of 34 ± 4 min. Further recovery in the twitch $P_{di}$ values occurred by 70 ± 4 min post-exercise where there were no significant differences from pre-exercise values.

M waves

M wave latency, time and rectified area are shown in Table 6. The time from stimulation of the phrenic nerves to the rise in electrical activity obtained from the diaphragmatic surface electrodes was unchanged following exercise, as was the...
Table 6. M wave characteristics before and after exercise at 95% of $\dot{V}_{\text{O}_2,\text{max}}$

<table>
<thead>
<tr>
<th></th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>6.40</td>
<td>6.34</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Time (ms)</td>
<td>48.34</td>
<td>51.94</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>1.71</td>
<td>1.81</td>
</tr>
<tr>
<td>Area (mV)</td>
<td>1.89</td>
<td>1.68</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>0.35</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Results are given as means ± s.e.m.; n = 5.

Latency, period of time from stimulation to initial electrical activity at the surface electrodes;

Time, period of time from onset of M wave electrical activity to no activity; Area, total rectified area under M wave curve.

* Significant difference between pre- and post-exercise values at $P < 0.05$.

duration of the M wave from each hemidiaphragm and the integrated area. Similar findings (not shown) were obtained in two subjects who had M wave values obtained from oesophageal EMGs.

**Diaphragmatic work vs. changes in stimulated $P_{di}$**

We used the mean change in stimulated $P_{di}$ at FRC with 1, 10 and 20 Hz as an index of diaphragm fatigue following exercise for each subject. The decrease in this index pre- vs. post-exercise at 95% of $\dot{V}_{\text{O}_2,\text{max}}$ averaged $20 \pm 3\%$ (range $-4$ to $-31\%$) and after the 85% exercise intensity, $15 \pm 5\%$ (range $+1\%$ to $-38\%$). We determined the relationship of our index of diaphragmatic fatigue to several parameters of diaphragmatic work during endurance exercise. The most significant correlation for both exercise conditions was with the percentage change in the $\int P_{di} \text{min}^{-1}$ from rest to the final 2 min of exercise ($r = -0.67$ to $-0.85$) (Fig. 8). Reductions in the fatigue index were also correlated with the $P_{di}$ reached during...
exercise relative to the available capacity for $P_{di}$ ($r = 0.52, P < 0.05$). No relationship was found with our fatigue index and an index of cumulative diaphragmatic work as determined by the $\int P_{di} \text{min}^{-1}$ over the entire exercise time.

During exercise, subjects showed variable changes in the $\int P_{di} \text{min}^{-1}$ over time (see Fig. 3). During the higher work intensity, we found a positive relationship between our fatigue index and the change in the $\int P_{di} \text{min}^{-1}$ over the duration of exercise ($r = -0.80, P < 0.01$) (see Fig. 9 left). That is, the greater the fall in the time integral for $P_{di}$ throughout exercise, the lesser the fall in stimulated $P_{di}$ following exercise.

Over the final minutes of each exercise intensity, there was considerable variability in work intensity (60–112% of $V_{O_2,\text{max}}$). When we combined both exercise loads we found a significant negative relationship between relative exercise intensity and the reduction in our index of diaphragmatic fatigue ($r = -0.67, P < 0.05$) (Fig. 9 right). Note that the likelihood of occurrence of diaphragm fatigue with exercise increased substantially when work intensity exceeded 85% of $V_{O_2,\text{max}}$.

**DISCUSSION**

The major finding of this investigation was that heavy, whole body endurance exercise causes diaphragmatic fatigue in spontaneously breathing healthy humans with a variety of fitness levels. We define 'fatigue' as a condition in which there is a reduction in the force-generating capacity of the muscle resulting from muscle activity under load which is reversible by rest (National Heart Lung Blood Institute, 1990). This was shown by significant declines in $P_{di}$ from pre- to post-exercise when the phrenic nerves were stimulated supramaximally and bilaterally with a twitch or
tetanically with frequencies of 10 and 20 Hz. Significant decreases in stimulated $P_{di}$ were observed following 14–30 min of endurance exercise over a range of exercise intensities (60–112% $V_{O_2,max}$). However, the likelihood of incurring highly significant diaphragmatic fatigue was higher in subjects who exercised at intensities in excess of 85% of $V_{O_2,max}$. Diaphragmatic fatigue was not relieved until after more than 1 h of recovery from exercise. The degree of fatigue, as assessed by the average decrease in the $P_{di}$ at all stimulation frequencies post-exercise, was significantly related to the percentage increase in diaphragmatic work (i.e. $\int P_{di} \, dt$) during exercise, and to the $P_{di}$ generated during exercise relative to the available capacity for $P_{di}$.

Potential sources of error

There are several potential sources of error with the BPNS technique (Bellemare & Bigland-Ritchie, 1984; Hubmayr, Litchy, Gay & Nelson, 1989; National Heart Lung Blood Institute, 1990). These include maintaining reproducible supramaximal nerve stimulation, assuring stimulation occurs at similar muscle lengths and the occurrence of ‘twitch potentiation’. We attempted to control each of these potential sources of error (also see Methods). Firstly, the excellent reproducibility of our M waves before and after exercise and the relatively low coefficient of variations for repeated stimulated $P_{di}$ measurements confirms the constancy of our supramaximal phrenic nerve stimulation. Secondly, we documented the constancy of lung volumes during all stimulations before and after exercise, which we presume to imply a constant diaphragmatic length. Thirdly, we ruled out the importance of any effect from twitch potentiation to our conclusions by showing similar effects of exercise on the stimulated $P_{di}$ obtained with both tetanic and twitch stimulations.

Finally, we note that any change in abdominal wall compliance induced by exercise will potentially affect the stimulated $P_{di}$. For example, if abdominal wall compliance increases, then as the diaphragm descends, in response to phrenic nerve stimulation, $P_g$ would be expected to be less and $P_{di}$ to fall. If abdominal wall compliance is reduced following exercise (perhaps secondary to continued expiratory abdominal muscle tonic activity) $P_g$ would be increased post-exercise in response to phrenic nerve stimulation. Speaking against these possibilities are our findings that, in most cases, the fall in $P_{di}$ was due to approximately equal or greater decreases in $P_o$ vs. $P_g$. Furthermore, in two subjects we used custom made fibreglass abdominal casts to constrain abdominal wall movement more consistently during stimulation before and after exercise. In both cases, similar findings were obtained with or without the cast, i.e. in one case no fatigue was noted and in the other case fatigue was present. Thus, while we cannot be certain that abdominal wall compliance was not altered following exhaustive exercise, we think it unlikely that such changes contribute significantly to the observed reductions in $P_{di}$ with BPNS.

Respiratory muscle fatigue during exercise

Locomotor muscle fatigue has been shown to occur after exhaustive running or uphill walking, by using supramaximal stimulation of motor nerves (Davies & White, 1982). To the contrary, as addressed in the Introduction, an objective demonstration of respiratory muscle fatigue due to whole body exercise has not been previously demonstrated.
EXERCISE-INDUCED DIAPHRAGMATIC FATIGUE

We believe our data using the BPNS technique offers the first objective quantitative evidence of end-organ diaphragmatic fatigue resulting from endurance whole body exercise in the healthy subject. Since we did not obtain our data during or immediately following exercise, we might have underestimated slightly the actual reduction in supramaximal stimulated $P_{di}$. There are several sites from the brain to the diaphragm that may contribute to this fatigue process (Jones, 1981; Bellemare & Bigland-Ritchie, 1987; Sieck & Fournier, 1990). These include so-called 'centrally' mediated inhibition, a failure in neural transmission and/or actual end-organ fatigue. However, our use of the BPNS technique permits us only to conclude with certainty that whole body endurance exercise caused end-organ fatigue i.e. at the level of the diaphragm. Neural transmission failure in the diaphragm may have occurred, as evidenced by M wave shape and timing changes during supramaximal stimulation. However, these changes commonly recover to normal very quickly and at a time when mechanical failure is still evident (Bellemare & Garzaniti, 1988; Thomas, Woods & Bigland-Ritchie, 1989). We would have missed these changes with our test protocol. We were not able to test directly for 'central' fatigue effects on $P_{di}$ during exercise, although we do have some indirect findings which indicate that this might be a factor (see below).

Several factors which may influence inspiratory muscle and diaphragmatic fatigue in humans have been identified from studies of volitional hyperpnoea, usually combined with resistive loading. These factors include the maximum available (static) $P_{di}$, and the magnitude of the tidal breathing $P_{di}$ developed relative to the capacity for pressure generation (Bellemare & Grassino, 1982), muscle length and velocity of shortening (McCool, McCann, Leith & Hoppin, 1986; Dodd, Collett & Engel, 1988), breathing frequency and diaphragmatic duty cycle (Bellemare & Grassino, 1982) and work rate of the muscle (mechanical work/breath multiplied by breathing frequency) (Collett, Perry & Engel, 1985; McCool et al. 1986). The unifying quantity common to all these mechanical determinants of respiratory muscle fatigue may be respiratory muscle $\dot{V}_O$, (McCool, Tzelepis, Leith & Hoppin, 1989).

During endurance exercise, our subjects reached a $\dot{V}_E$ of 150 l min$^{-1}$ over the last 4–5 min of exercise at 95% of $\dot{V}_O_{max}$ and 126 l min$^{-1}$ during exercise at 85% of $\dot{V}_O_{max}$. High tidal volumes (2.6–2.9 l) were sustained throughout exercise, and a slowly rising EELV occurred over the latter stages as the degree of expiratory flow limitation increased in magnitude. This resulted in a gradual increase in end-inspiratory lung volumes, which averaged > 85% of TLC over the final few minutes of exercise. Thus, a substantial elastic load was presented to the inspiratory muscles during heavy endurance exercise. Furthermore, velocity of inspiratory muscle shortening was also greatly increased as indicated by peak inspiratory flow rates which were 8–10 × resting levels at end-exercise. The peak $P_{di}$ achieved during tidal inspiration at end-exercise averaged 60% (range 35–85%) of the available (dynamic) $P_{di, max}$. This coincided with 300–500% increases in the $fP_{di}$ min$^{-1}$ which were maintained for 10–30 min. Therefore, during prolonged heavy exercise the diaphragm and other inspiratory muscles are required to produce and sustain a substantial hyperpnoea under conditions of high elastic loads and velocity of muscle shortening, working at large percentages of their available capacity for inspiratory muscle pressure generation. Recent evidence suggests that as $\dot{V}_E$ exceeds 120 l min$^{-1}$
and expiratory flow rate becomes increasingly limited, marked increases occur in the O₂ cost of breathing, requiring as much as 12–16% of whole body V̇O₂ max (Aaron et al. 1992). These data are consistent with our finding of a significant correlation between the magnitude of the increase in the integral of Pdi over time from rest to the end of exercise and the magnitude of fatigue determined following exercise. Thus the amount of work done by the diaphragm in producing exercise hyperpnoea appears to be at least one of the major factors in producing fatigue. On the other hand, we cannot explain the finding that the cumulative work performed by the diaphragm during exercise was not correlated significantly with the fatigue incurred. This might imply that the diaphragm experienced significant fatigue relatively early in the exercise period (see below).

It was of interest that our subjects demonstrated a parallel fall in stimulated Pdi values at the different lung volumes. As a result the percentage decline in twitch Pdi increased as lung volume increased. We interpret this to mean that the diaphragmatic fatigue produced may be greater the shorter the diaphragmatic length; in turn, this finding may be related to how muscle fibres were recruited to perform the ventilatory task. During tidal breathing in heavy exercise, force development by the diaphragm was greatest at approximately 80% of TLC; however, this was also the lung volume where the capacity for force development was the most reduced. In addition, the flow rate was at a peak at this same lung volume, so that the velocity of muscle shortening was also near maximal levels, which further reduced the capacity of the muscle for pressure development. Therefore this may be the lung volume, or diaphragmatic length, where the need for recruiting more fibres and faster (i.e. more fatiguable) fibres is greatest. In turn, this requirement would contribute to a greater decrease in Pdi in response to BPNS at this lung volume.

In addition to the magnitude of the increases in diaphragmatic work achieved during exercise as a determinant of fatigue, it is also feasible that the metabolic end products of locomotor muscle contraction during prolonged exercise could precipitate or at least contribute to diaphragmatic fatigue. Indeed, experimentally induced metabolic acidosis has been shown to cause reduced twitch tension in the diaphragm in the anaesthetized animal (Fitzgerald, Hauer, Bierkamper & Raff, 1984), even in the absence of augmented muscle contraction. In turn, it is likely that a significant metabolic acidosis in arterial plasma did prevail during most of the exercise period and over at least a significant portion of the recovery period in most of our exercise trials requiring greater than 80% of V̇O₂ max (Hanson, Claremont, Dempsey & Reddan, 1982). Very high concentrations of lactic acid have also been shown to accumulate in the diaphragm of the exercising rat, even in the absence of significant glycogen depletion (Fregosi & Dempsey, 1986). Our current study does not permit us to identify any independent effects of the exercise-induced changes in metabolic end-products on diaphragmatic fatigue. In this regard it would be instructive to determine the effects of exercise on force development in a non-exercising, i.e. ‘resting’ muscle, exposed to an extracellular fluid environment similar to that seen by the diaphragm.

In summary, our findings demonstrate that whole body endurance exercise in excess of 85% V̇O₂ max will indeed cause significant, sustained fatigue of the diaphragm at the end-organ level in healthy subjects with a wide variation in fitness levels. Our
data do not relate specifically to the duration of exercise required to produce significant diaphragmatic fatigue, but the combined results of ourselves using endurance-type exercise with the largely negative findings of Levine & Henson (1988) using a short-term progressive exercise test to \( \dot{V}_{O_2, \text{max}} \), do suggest that the high intensity exercise must be sustained for at least 10 min. In turn, we believe these high intensities of endurance exercise cause diaphragmatic fatigue because they require high levels of sustained ventilatory output (i.e. \( \dot{V}_E > 125 \, \text{l min}^{-1} \)), diaphragmatic work (more than four to five times resting levels) and respiratory muscle \( \dot{V}_{O_2} \), and accumulation of metabolic end-products from contracting locomotor muscles. While these intensities of exercise and levels of respiratory muscle energy expenditure are substantial, they are clearly achievable in response to a physiological stimulus even in the relatively untrained healthy subject.

**Implications for the response to exercise**

What effect does the fatigue expressed by an average fall of 20% of the supramaximally stimulated diaphragm have on the ventilatory response to exercise? First, it is unlikely that exercise-induced diaphragmatic fatigue substantially affects the adequacy of the overall ventilatory response to long-term exercise. Indeed, even those of our subjects who increased \( \int P_{di} \, \text{min}^{-1} \) the most during exercise, who exceeded 80% of peak \( P_{cap,di} \) during exercise and who showed reductions of > 30% in their (post-exercise) supramaximally stimulated \( P_{di} \), showed a progressive hyperventilation during prolonged exercise.

On the other hand, some of our data do suggest that the pattern of respiratory muscle recruitment during heavy exercise may be affected by exercise-induced diaphragmatic fatigue. We observed that, beginning at the fifth to tenth minute of each exercise period, the \( \int P_{di} \, \text{min}^{-1} \) tended to plateau for the remainder of the exercise period at a time when \( \dot{V}_E \), inspiratory flow rate and the \( \int P_e \, \text{min}^{-1} \) all continued to rise substantially until exercise terminated. These data suggest that the diaphragm is contributing less, and the ‘accessory’ inspiratory (and expiratory) muscles more, to the production of the hyperventilatory response as the duration of exercise proceeds. Furthermore, those subjects who decreased or minimized the relative contribution of the diaphragm to total ventilatory work the most over the duration of exercise, showed less fatigue of the diaphragm in the post-exercise period. Perhaps these observations might be due to the onset of fatigue in the diaphragm during the early period of heavy exercise, which reflexly inhibits further diaphragmatic recruitment and triggers the recruitment of additional inspiratory (and expiratory) muscles (Jammes, Buchler, Delpierre, Rasidakis, Grimaud & Roussos, 1986). The result is that the hyperpnoea may proceed without further fatigue of the primary inspiratory muscle. This postulate needs testing, perhaps first by using BPNS to determine whether the initial failure (or ‘unwillingness’) of \( P_{di} \) to continue to increase commensurate with increasing ventilatory unwillingness as exercise continues, does indeed coincide with some measurable degree of actual, end-organ diaphragmatic fatigue.

We are indebted to David (Murray) Pegelow for his technical assistance and to the subjects for their perseverance. We also thank Peter Eichman and Kurt Saupe for assistance with development of the BPNS technique, Francois Bellemare for his helpful advice on application of BPNS and
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