

Diaphragm fatigue during exercise at high altitude: the role of hypoxia and workload

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ABSTRACT: The effect of high altitude (HA) on exercise-induced diaphragm fatigue in normal subjects was examined.

Eight normal subjects completed an incremental exercise test at sea level (SL) and at 3,325 m. Before (baseline), during, and after exercise (recovery), maximal transdiaphragm pressure ($P_{di,sniff}$), breathing pattern, and diaphragmatic effort (PTP_{di}) were measured. Arterialized blood lactate was measured at baseline and during recovery.

At maximal exercise (WR_{max}) $P_{di,sniff}$ fell to 72% and 61% of baseline at SL and HA respectively, recovering to baseline in 60 min at SL, and >60 min at HA. At the 5th min of recovery, circulating lactate was six-fold and seven-fold baseline at SL and HA, respectively. The time course of circulating lactate recovery was as for $P_{di,sniff}$. At WR_{max} PTP_{di} was 80.74 ± 9.87 kPa·s⁻¹ at SL and 64.13 ± 8.21 kPa·s⁻¹ at HA. HA WR_{max} compared to isowork rate, SL data showed a lower $P_{di,sniff}$ (8.90 ± 0.68 versus 11.24 ± 0.59 kPa) and higher minute ventilation (117 ± 11 versus 91 ± 13 L·min⁻¹), PTP_{di} being equal.

To conclude, in normal subjects hypoxia-related effects, and not an increase in diaphragm work, hastens exercise-induced diaphragm fatigue and delays its recovery at high altitude compared to sea level.

Eur Respir J 2001; 17: 674–680.

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Keywords: Diaphragm fatigue, exercise testing, hypoxia, respiratory mechanics, respiratory muscles

Received: March 27 2000
Accepted after revision November 23 2000

Exercise limitation at high altitude (HA) has been extensively studied using sophisticated techniques (Swan-Ganz catheterization, inert gas diffusion, *etc.*) [1, 2]. However, data concerning respiratory muscle function are scanty nevertheless, exercise on either cycle-ergometer or treadmill at sea level (SL) has been shown to produce diaphragm fatigue in healthy subjects [3–6] as measured with volitional (Mueller and sniff) [3, 4, 6] and objective (bilateral phrenic nerve stimulation; BPNS) manoeuvres [3–6]. Therefore, the aim was to investigate exercise-induced diaphragm fatigue at HA. HA might well resemble normobaric hypoxia which is known to hasten diaphragm fatigue during exercise [3]. However, at HA the density of air decreases, resulting in decreased airway flow resistance [7] and reduced work of breathing. The authors sought to identify possible different physiological responses to exercise at HA compared to normobaric hypoxia [3].

Methods

Subjects

Eight healthy male subjects (age 38 ± 2 yrs) born and living at SL, not acclimatized to HA, gave their consent to perform the study.

Measurements

Flow (V') was measured with a mass flow sensor (Sensormedics, Yorba Linda, CA, USA) and volume (V) was obtained by integrating the flow signals. The mass flow sensor was calibrated with a 3 L syringe before each test.

Oesophageal (P_{oes}) and gastric (P_{ga}) pressure changes were measured as previously reported [8]. Airway opening pressure (P_{ao}) was measured *via* a side-port placed on a two-way breathing valve (720068, Jaeger, Würzburg, Germany) connected to the mass flow sensor. Transpulmonary (P_L) and transdiaphragmatic (P_{di}) pressures were obtained by subtracting P_{oes} from P_{ao} and P_{ga} , respectively. P_L , V' and V data were used to calculate dynamic lung elastance (EL_{dyn}) and lung resistance (R_L) [9].

Maximal oesophageal and transdiaphragmatic pressures were measured with the Mueller [10] ($P_{oes,max}$ and $P_{di,max}$, respectively) and the sniff [10] ($P_{oes,sniff}$ and $P_{di,sniff}$, respectively) manoeuvres. End-expiratory lung volume (EELV) before sniffs was monitored using end-expiratory P_{oes} [11, 12]. To assess diaphragmatic length, P_{ga} was continuously monitored [11]. Criteria for accepting and correcting $P_{oes,sniff}$ for abdominal muscle contractions ($P_{oes,sniff,corr}$) were those described by KYROUSSIS *et al.* [12]. The sniff showing the greatest

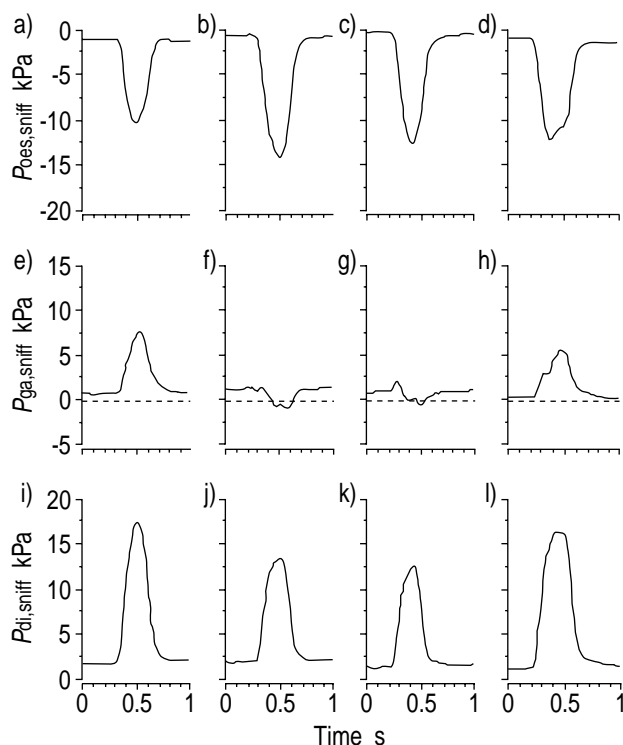


Fig. 1. – Representative recordings of maximal inspiratory pressures measured with the sniff manoeuvre in subject 6 at SL. a-d) maximal inspiratory oesophageal ($P_{oes,sniff}$), e-h) gastric ($P_{ga,sniff}$) and i-l) transdiaphragmatic ($P_{di,sniff}$) pressures, measured at baseline (a, e, i), maximal exercise (b, f, j), and at the 1st min (c, g, k) and 60th min of recovery (d, h, l).

pressure deflection in each condition and fulfilling all criteria [12] was selected for analysis. Figure 1 shows a representative record of sniffs during the protocol.

To estimate the diaphragmatic and respiratory muscle effort, the pressure-time product for diaphragm (PTP_{di}) and respiratory muscles (PTP_{oes}) over 1 min were computed [8]. Since diaphragm external work rate (W'_{di}) has been shown to be a better index of diaphragm effort than PTP_{di} when inspiratory flow is not constant [13], the former was also computed, by multiplying PTP_{di} by the actual mean inspiratory flow [13].

Oxygen consumption ($V'O_2$), and carbon dioxide production ($V'CO_2$) were measured breath-by-breath (Vmax29; Sormedics, Yorba Linda, CA, USA). Gas analysers were calibrated before each test. Heart rate (HR) was measured from a 12-lead electrocardiogram.

Arterialized venous blood [14] was obtained to estimate P_{a,CO_2} (ABL 300; Radiometer, Copenhagen, Denmark) and measure lactate (2300 STAT; Yellow Spring Instrument, Yellow Spring, OH, USA). P_{a,CO_2} and barometric pressure (P_B) were used to calculate the alveolar oxygen partial pressure (P_{A,O_2}) [15]. A finger oximeter (BIOX 3760; Datex-Ohmeda, Division Instrumentarium Corp., Helsinki, Finland) measured arterial O_2 saturation (S_{a,O_2}).

Protocol

Measurements were performed at baseline, during an incremental exercise test to volitional tolerance on

cycle-ergometer (Ergo-metrics 800S; Sormedics, Yorba Linda, CA, USA) and during the hour after exercise. The protocol was performed both at 3,325 m of altitude (Rifugio Torino, Monte Bianco, Italy) and at SL. At HA, P_B and resting P_{A,O_2} were 66.93 ± 0.14 and 7.60 ± 0.27 kPa respectively. At HA, the subjects were studied within a few hours of the ascent (cableway), sitting on the cycle-ergometer with the thorax kept in the same position throughout the study.

Baseline measurements. Before exercise, spirometry showed normal values [16] in every instance. The sniff and Mueller manoeuvres were practised until both appeared reproducible ($\pm 5\%$) and fulfilled all inclusion criteria. Then subjects started to breathe through the mouthpiece until they felt comfortable with the measurement apparatus. At this point, breathing pattern was recorded for 1 min, followed by measurements of HR, blood samples, and maximal inspiratory pressures.

Exercise. After 3 min of unloaded pedalling, the work rate (WR) was increased by $20\text{--}30$ watts \cdot min $^{-1}$ to volitional tolerance and/or until maximal predicted HR ($220 - \text{age}$) was reached [15]. Incremental loads differed between subjects to keep the exercise within 10 min [15]. In the last 30 s of unloaded pedalling and between the 30th and 45th second of every minute during exercise, $V'O_2$, $V'CO_2$, HR and breathing pattern were measured; the last 15 s of each minute were used to perform sniffs (mouthpiece off, mouth closed). S_{a,O_2} was measured throughout exercise.

Recovery. After exercise, the breathing pattern and sniffs were recorded each minute for the first five min and at the 10th, 15th, 30th, 45th and 60th min. The Mueller manoeuvre was performed and arterialized blood samples were obtained starting from the fifth min.

Additional experiments

On a separate day, four subjects repeated the exercise at SL using cervical magnetic stimulation (CMS) to obtain twitch maximal transdiaphragmatic pressure ($P_{di,tw}$) and the degree of voluntary neural activation during maximal voluntary efforts by the twitch occlusion technique [17]. Right and left diaphragmatic electromyograms (EMEdi) were obtained using surface electrodes (Ref. 9013L0202; Dantec Medical, Tonsbakken, Denmark) connected to an electromyograph (Mystro+ MS20, Medelec, Woking, Surrey, UK) [18], amplified and band pass filtered (band width 20 Hz–5 kHz).

CMS was performed by a Magstim 200 stimulator equipped with a circular (doughnut shaped) 90 mm coil with a maximum magnetic field of 2.3 Tesla (Magstim, Whitland, Dyfed, UK) [18, 19]. Stimulation amplitude was 100% of the maximal output of the stimulator. Supramaximal stimulation was verified according to SIMILOWSKI *et al.* [19]. Stimuli were delivered at end-expiration with the airways closed.

$P_{di,tw}$ and, consecutively, twitch occlusion were performed firstly 10 min after end-exercise to avoid twitch potentiation, and then after 60 min of recovery.

Data and statistical analysis

All data are reported as mean \pm SEM. A paired t-test [20] was used to compare data, *i.e.* maximal WR (WR_{max}), iso-WR data at SL and HA, and for comparison between $P_{di,max}$ and $P_{di,sniff}$. One-way analysis of variance (ANOVA) [20] was used for determination of differences in mean values for $P_{di,sniff}$, lactate and $\dot{V}E$ during baseline, exercise and recovery, and, when allowed by the F-value, the significance between measures was computed using Fisher's Protected least significant difference (PLSD) test. Significance was set at $p<0.05$.

Results

S_{a,O_2} , P_{a,CO_2} , and pH data are shown in table 1. At SL, pH became acidotic at WR_{max} despite the marked hyperventilation (decrease in P_{a,CO_2}). S_{a,O_2} remained constant throughout exercise. At HA, all subjects showed hypocapnia and hypoxaemia ($S_{a,O_2}<90\%$) at baseline; both increased at maximal exercise.

$P_{di,sniff}$ and $P_{oes,sniff}$ were compared to $P_{di,max}$ and $P_{oes,max}$, respectively, and no difference was found. Therefore only data based on the sniff manoeuvre is reported.

Maximal exercise

Table 2 shows WR, metabolic parameters, HR, and breathing pattern at maximal exercise. WR_{max} was similar at SL and HA, even though three subjects reached a lower WR at HA. At SL the subjects reached predicted maximal $\dot{V}O_2$ ($\dot{V}O_{2,max}$) and HR (HR_{max}). At HA both $\dot{V}O_{2,max}$ and HR_{max} were lower (12% and 6%, respectively), though maximal $\dot{V}CO_2$ ($\dot{V}CO_{2,max}$) was similar. EL was similar at SL and HA (table 1). RL was lower at HA compared to SL (-11%, table 1). The breathing pattern at WR_{max} was similar at SL and HA. Figure 2a shows average $P_{di,sniff}$ values. $P_{di,sniff}$ was lower at WR_{max} compared to baseline (SL: average 72%, range 61–80%; HA: average 61%, range 37–80%) in all but one test at SL (94%). $P_{di,sniff}$ had a positive gastric component ($P_{di,sniff}>P_{oes,sniff}$) before exercise in all instances. By contrast, at WR_{max} , the gastric component of $P_{di,sniff}$ became always negative ($P_{di,sniff}<P_{oes,sniff}$) at HA, while at SL it was negative in six

Table 2. – Measurements during maximal exercise at sea level and high altitude

Variable	SL	HA
WR_{max} watts	221 \pm 22	205 \pm 14
$\dot{V}O_{2,max}$ mL \cdot min $^{-1}$	2868 \pm 304	2525 \pm 207*
$\dot{V}O_{2,max}$ % pred	101 \pm 10	90 \pm 6*
$\dot{V}CO_{2,max}$ mL \cdot min $^{-1}$	3411 \pm 387	3142 \pm 257
HR_{max} beats \cdot min $^{-1}$	179 \pm 2	169 \pm 4*
HR_{max} % pred	98 \pm 1	93 \pm 2*
$\dot{V}E_{max}$ L \cdot min $^{-1}$	87.7 \pm 12.3	98.0 \pm 11.6
f_{max} breaths \cdot min $^{-1}$	33.0 \pm 2.3	35.9 \pm 3.1
$\dot{V}T_{max}$ L	2.59 \pm 0.26	2.72 \pm 0.25

Values are mean \pm SEM. WR_{max} : maximal work rate; $\dot{V}O_{2,max}$: maximal oxygen consumption; $\dot{V}CO_{2,max}$: maximal carbon dioxide production; HR_{max} : maximal heart rate; $\dot{V}E_{max}$: minute ventilation during maximal exercise; f_{max} : breathing frequency during maximal exercise; $\dot{V}T_{max}$: tidal volume during maximal exercise. *: $p<0.05$ SL versus HA.

subjects and reduced, but still positive in the remaining two. At SL but not at HA $P_{di,sniff}$ recovered to baseline within 60 min.

At SL, $P_{oes,sniff}$ was higher at WR_{max} (11.34 \pm 0.49 kPa) than at baseline (9.97 \pm 0.39 kPa), whereas at HA it was equal (WR_{max} : 10.36 \pm 0.78 kPa; baseline: 8.99 \pm 0.29 kPa). $P_{oes,sniff}$ corr showed similar results at WR_{max} (SL: 11.24 \pm 0.39 kPa; HA: 10.17 \pm 0.68 kPa).

At SL, PTP $_{di}$ averaged 80.74 \pm 9.87 kPa \cdot s $^{-1}$ over 1 min at WR_{max} , all subjects being on or above the fatigue threshold of 53.76–58.65 kPa \cdot s $^{-1}$ over 1 min [4]. PTP $_{di}$ at WR_{max} was lower at HA than at SL (64.13 \pm 8.21 kPa \cdot s $^{-1}$ over 1 min), but five subjects were still above and three lay just below the fatigue threshold (42.03–44.97 kPa \cdot s $^{-1}$ over 1 min). PTP $_{di}$ /PTP $_{oes}$ ratio fell below unity at WR_{max} both at SL and at HA (but was lower at HA), and recovered to baseline within 5 min in both conditions (fig. 2b).

At SL, lactate increased from 0.9 \pm 0.1 mEq \cdot L $^{-1}$ at rest to 6.2 \pm 0.7 mEq \cdot L $^{-1}$ at the 5th min of recovery, regaining baseline at 60 min (1.0 \pm 0.1 mEq \cdot L $^{-1}$). At HA, lactate increased from baseline (0.9 \pm 0.1 mEq \cdot L $^{-1}$) to the 5th min of recovery (6.7 \pm 0.6 mEq \cdot L $^{-1}$) but, as with $P_{di,sniff}$, lactate did not recover to baseline within 60 min (1.3 \pm 0.2 mEq \cdot L $^{-1}$, $p<0.05$). In contrast to $P_{di,sniff}$ and lactate, $\dot{V}E$ reached baseline ($\dot{V}E$ at SL: 12.8 \pm 1.6 L \cdot min $^{-1}$; $\dot{V}E$ at HA: 15.3 \pm 2.3 L \cdot min $^{-1}$) within 15 min at SL (15.2 \pm 2.4 L \cdot min $^{-1}$) and within 30 min at HA (14.9 \pm 1.5 L \cdot min $^{-1}$).

Table 1. – Percentage of O_2 saturation of arterial blood (S_{a,O_2}) as estimated by pulse oximetry, arterialized blood pH and CO_2 partial pressure (P_{a,CO_2}), dynamic lung elastance (EL_{dyn}), and pulmonary resistance (RL) for normoxic (sea level; SL) and hypoxic (high altitude; HA) exercise

Variable	SL		HA	
	Baseline	Max	Baseline	Max
S_{a,O_2} %	96.3 \pm 0.4	96.1 \pm 0.5	87.5 \pm 1.0(**)	83.0 \pm 0.9**
pH	7.407 \pm 0.004	7.269 \pm 0.013**	7.469 \pm 0.005(**)	7.327 \pm 0.011**
P_{a,CO_2} kPa	5.25 \pm 0.13	4.60 \pm 0.25**	4.59 \pm 0.15(**)	3.80 \pm 0.17**
EL_{dyn} kPa \cdot L $^{-1}$	0.58 \pm 0.06		0.65 \pm 0.06	
RL kPa \cdot L $^{-1}$ \cdot s	0.20 \pm 0.03		0.18 \pm 0.02	

Values are presented as mean \pm SEM. Baseline: measurements taken before exercise; Max: measurements taken at maximal exercise. **: $p<0.01$ max versus baseline; (baseline at HA versus baseline at SL).

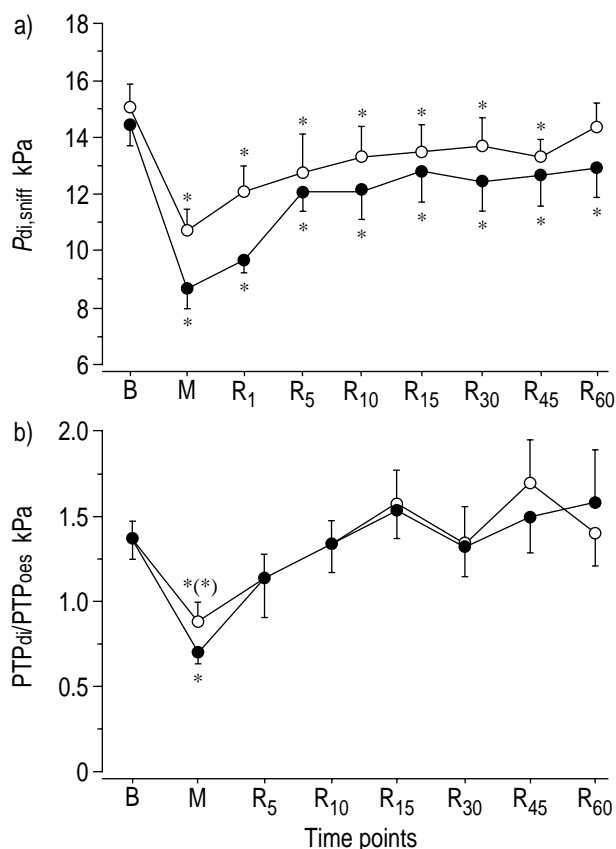


Fig. 2. – Mean \pm SEM of: a) the maximal transdiaphragm pressure ($P_{di,sniff}$), and of b) the pressure-time product for the diaphragm expressed as a fraction of the oesophageal pressure-time product (PTP_{di}/PTP_{oes}) at baseline (B), maximal exercise (M), and during 60 min of recovery (R₁-R₆₀) measured at sea level (○) and at high altitude (●). During maximal exercise the PTP_{di}/PTP_{oes} ratio dropped below unity indicating that the majority of the work of breathing had been shifted from the diaphragm to the accessory inspiratory muscles. *: $p < 0.05$ condition versus B. (sea level versus high altitude)

Isowork rate

WR_{max} was lower at HA in three subjects. To data at isowork rate conditions (iso-WR) compared in each subject, WR_{max} at HA was matched in these three subjects with a similar WR obtained at SL. At iso-WR, $P_{di,sniff}$ was significantly lower at HA (fig. 3a), $P_{oes,sniff}$ never being below baseline (fig. 3b). $V'E$ was higher at HA (117.2 ± 11.4 L \cdot min $^{-1}$) than at iso-WR SL (91.3 ± 13.3 L \cdot min $^{-1}$) because of higher frequency (35.9 ± 3.1 and 30.6 ± 2.1 breaths \cdot min $^{-1}$, respectively, $p < 0.05$). Similar results were obtained for mean inspiratory flow (WR_{max} HA: 3.64 ± 0.41 L \cdot s $^{-1}$; iso-WR SL: 3.03 ± 0.29 L \cdot s $^{-1}$, $p < 0.05$). Notwithstanding the higher $V'E$ at HA, PTP_{di} and W'_{di} were similar at iso-WR (fig. 4).

Volitional versus objective measurements

In four subjects retested at SL, both $P_{di,tw}$ and $P_{di,sniff}$ decreased significantly 10 min after exercise, and recovered 60 min after exercise (table 3). All subjects showed near complete activation of the

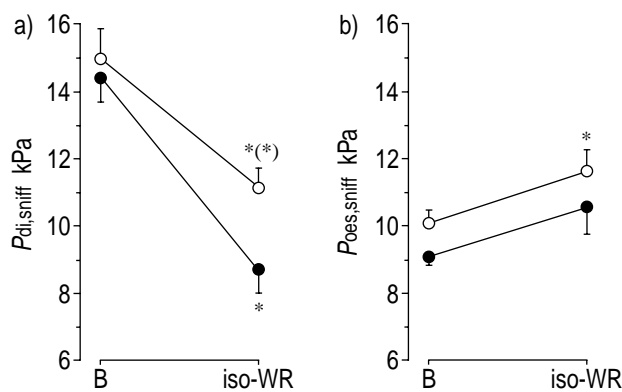


Fig. 3. – Mean \pm SEM of: a) maximal transdiaphragm ($P_{di,sniff}$) and b) oesophageal ($P_{oes,sniff}$) pressures measured at baseline (B) and at isowork rate (iso-WR) at sea level (SL; ○) and at high altitude (HA; ●). Iso-WR: workload at SL matched to maximal workload reached at HA. The reduction of $P_{di,sniff}$ at iso-WR with respect to baseline was greater at HA. By contrast, $P_{oes,sniff}$ increased slightly both at SL and HA at iso-WR. *: $p < 0.05$ B versus iso-WR (SL versus HA).

diaphragm, as no significant increase of P_{di} due to CMS was found (fig. 5). $P_{di,sniff}$ was similar to $P_{di,max}$ in any condition (fig. 5), showing near complete activation of the diaphragm also with this manoeuvre.

Discussion

The present data show that diaphragmatic force generating capacity (FGC) at maximal whole-body exercise was impaired at HA compared to equivalent work rates at SL, hypoxia alone being the predominant causative factor. This study also shows that, similarly to endurance exercise [3, 4], exhaustive incremental exercise can produce diaphragm fatigue at both SL and HA.

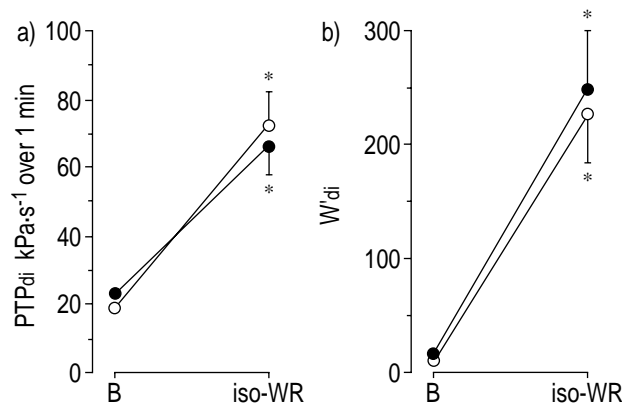


Fig. 4. – Mean \pm SEM of: a) pressure-time product for the diaphragm (PTP_{di}) and of b) the mechanical work performed by the diaphragm (W'_{di}). W'_{di} is the product of PTP_{di} and mean inspiratory flow ($V'I/t$). These parameters are shown at baseline (B) and at isowork rate (iso-WR) measured at sea level (SL; ○) and at high altitude (HA; ●). Iso-WR: workload at SL matched to maximal workload reached at HA. PTP_{di} was similar at SL and HA at both B and iso-WR. The same finding applied to W'_{di} which takes into account changes in diaphragm effort induced by flow rate variations. These data indicate that the effort of the diaphragm at iso-WR was similar both at SL and HA. *: $p < 0.05$ B versus iso-WR.

Table 3. – Volitional and objective measurements of diaphragmatic performance before and after exercise at sea level in four subjects

Variable	Baseline	Recovery 10 min	Recovery 60 min
$P_{di,sniff}$ kPa	15.15±0.41	11.09±0.75*	14.23±0.83
$P_{di,tw}$ kPa	1.93±0.21	1.41±0.16*	1.78±0.17

Values are mean±SEM. $P_{di,sniff}$: maximal transdiaphragmatic pressure obtained with a sniff manoeuvre; $P_{di,tw}$: maximal transdiaphragmatic pressure obtained with supramaximal cervical magnetic stimulation (single twitch). *: $p < 0.005$ condition versus baseline.

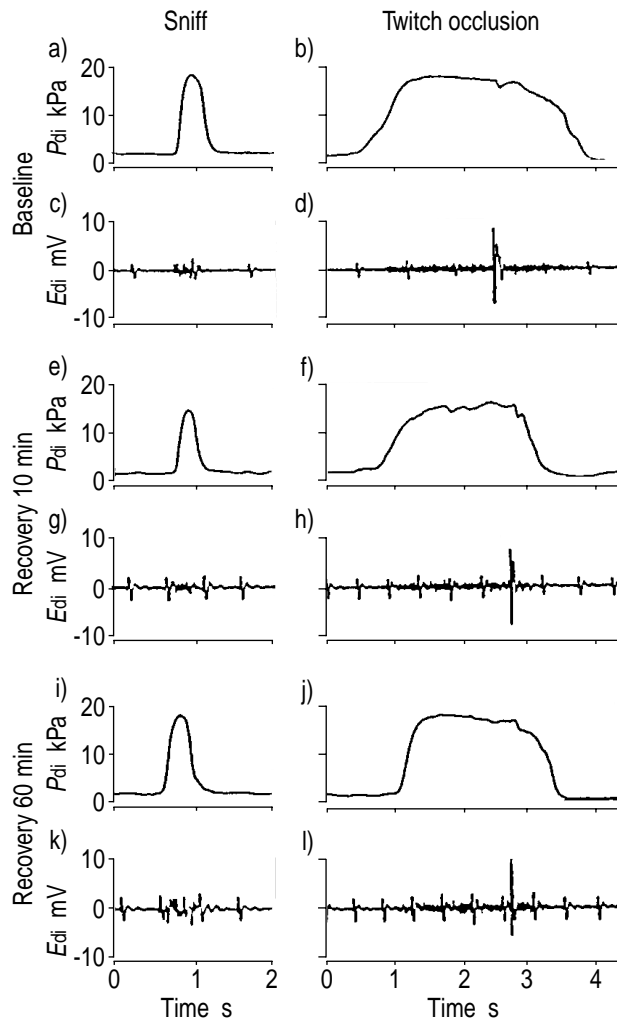


Fig. 5. – Maximal transdiaphragmatic pressure obtained with a "sniff" manoeuvre (a, c, e, g, i, k) and with a Mueller manoeuvre to which a single supramaximal twitch was superimposed (b, d, f, h, j, l) in a representative healthy subject at rest (a–d), at 10 min (e–h), and at 60 min (i–l) after the end of an exhaustive incremental exercise on cycle-ergometer performed at sea level. Electromyograms of the right hemidiaphragm in response to supramaximal cervical phrenic nerve stimulation (EMEdi) are also provided. The superimposition of supramaximal magnetic stimulation (indicated by the electrical artefact in the EMEdi tracing) on maximal quasistatic efforts never evoked a further increase in maximal transdiaphragmatic pressure. Note that P_{di} obtained with the "sniff" manoeuvre was similar to that obtained during the twitch occlusion test in every condition.

Technical considerations

BPNS is considered to have the best potential as a diagnostic test [21]. However, the sniff manoeuvre was primarily used because it can be performed during exercise, at variance with BPNS that requires preparation (5–8 min) after exercise [3].

Poor subject effort might impair the sniff manoeuvre [21]. All subjects had previous experience in performing the manoeuvre and were very well motivated. Moreover, sniff intrasubject variability was low (mean coefficient of variation at baseline: $2.3 \pm 1.0\%$, range: 0.1–5.9%). Tachypnoea and general exhaustion could limit $P_{di,sniff}$ performance [21]. However, $P_{di,sniff}$ recovered to baseline later than the breathing pattern, both at SL and HA. Furthermore, in contrast to $P_{di,sniff}$, $P_{oes,sniff}$ did not decrease at maximal exercise, suggesting maximal inspiratory effort even in these extreme conditions.

$P_{di,sniff}$ depends on lung volume [22]. The end-expiratory P_{oes} was monitored to ensure that sniff manoeuvres were performed at similar EELV, end-expiratory P_{oes} being within 0.15 kPa of baseline just before sniffs in any tested condition. Similar results were obtained with end-expiratory P_{ga} . These data suggest that diaphragmatic length was reasonably constant before $P_{di,sniff}$ manoeuvres.

Finally, according to TRAVALINE *et al.* [23], $P_{di,sniff}$ and $P_{di,max}$ reliability was tested at SL by CMS in a subset of four subjects on a separate day. The trend of $P_{di,sniff}$ decay was similar in the two tests, $P_{di,sniff}$ being within $\pm 5\%$ at each workload tested. Similarly to $P_{di,sniff}$, $P_{di,tw}$ was on average 27% lower than baseline 10 min after exercise (table 3), suggesting that $P_{di,sniff}$ was accurate in detecting losses of diaphragm FGC, at least in the condition tested. It was extrapolated that similar results could be obtained at HA.

Diaphragm fatigue

A decreased diaphragm FGC was found at maximal exercise both at SL and HA, which recovered to baseline late after exercise (fig. 2a), finding that fits the accepted definition of muscle fatigue [21]. These data extend to exhaustive incremental exercise, the evidence that diaphragm fatigue can be generated in normal subjects by endurance exercise [4–6, 24].

At end-exercise, $P_{di,sniff}$ decreased, whereas $P_{oes,sniff}$ did not or increased compared to baseline (figs. 1 and 3). These data are consistent with data obtained at, for example, the first minute of recovery (fig. 1). It may be argued that this observation cannot be explained by fatigue, since both the diaphragm and the extradiaphragmatic muscles serve to reduce the P_{oes} . However, $P_{di,sniff}$ was not only reduced compared to baseline, but it also became lower than $P_{oes,sniff}$ at end-exercise in almost all the conditions tested (see previously). This suggests diaphragm displacement into the rib cage during maximal manoeuvres, since end-expiratory abdominal pressure (P_{ab}) was close to baseline values at the beginning of the $P_{di,sniff}$ manoeuvre (*i.e.* abdominal muscles phasically inactive). Finally, prolonged forced muscle contractions (similar to those

developed by the respiratory muscles during exercise) can cause the phenomenon of potentiation [25]. In this condition, a $P_{oes,sniff}$ increase at end-exercise would be expected, but not a decrease in $P_{di,sniff}$ (rather it should increase too).

The presented finding contrasts with LEVINE *et al.* [26] who reported, in preliminary experiments, no sign of diaphragmatic fatigue at end-incremental exercise. However, they provided neither references nor data to support their statement. Short incremental exercise may appear too light to produce diaphragm fatigue. However, increased diaphragm work generated by sustained hyperpnoea at rest, comparable to that found in the present study, has been demonstrated to cause significant diaphragm fatigue [4]. Moreover, end-exercise pH and circulating lactate concentrations similar to those found in the present study have been shown to increase the amount of hyperpnoea-induced diaphragm fatigue [4].

Central, transmission and contractile fatigue have been described [10]. In the four subjects in whom the twitch occlusion technique [17] was performed, no signs of central fatigue were detected at least at SL (fig. 5). In the same subjects the M-wave was preserved after exercise, indicating absence of transmission fatigue in the condition tested. Finally, the long lasting duration of $P_{di,sniff}$ decay after exercise at SL and HA (fig. 2), and the postexercise reduction in twitch pressure observed at SL, suggests that contractile fatigue did occur [3, 4, 11].

Effects of high altitude on diaphragm fatigue

Incremental exhaustive exercise at 3,325 m altitude worsened the FGC compared to iso-WR SL (fig. 3) notwithstanding similar effort of the diaphragm (fig. 4). At HA, significant hypoxia was found. Comparable hypoxaemia has been shown to worsen exercise-induced diaphragm fatigue in normobaric conditions [3]. Increased diaphragm work was also found during hypoxic exercise at SL, suggesting that its combination with decreased O_2 transport impairs diaphragm FGC in this condition [3]. By contrast, it was found that, although V'_E was higher at HA than at iso-WR SL, PTP_{di} as well as W'_{di} (fig. 4) and PTP_{oes} were equal, indicating that in the experimental condition hypoxia *per se* hastened exercise-induced diaphragm fatigue.

EELV changes affect inspiratory muscle effort estimation [13]. EELV was not directly assessed, but it is generally accepted that in young healthy subjects it does not exceed its baseline value during exercise [27]. Conversely, the lower density of the air at HA (resulting in decreased airway flow resistance) could explain the increased inspiratory muscle efficiency in generating V'_E [7]. Indeed, similarly to previous studies [7], R_L decreased at HA approaching predicted values [28].

Not only did hypoxia impair independently exercise-induced diaphragm fatigue at HA, but it also influenced respiratory muscle interaction during exercise. ALIVERTI *et al.* [29] have shown that rib cage and abdominal muscles are progressively activated during exercise, allowing the diaphragm to act as a flow generator. Similar results were obtained in the present study at SL.

Indeed, PTP_{di}/PTP_{oes} ratio fell below unity at maximal exercise, indicating a strong activation of extra-diaphragmatic respiratory muscles (fig. 2b). At HA, end-exercise PTP_{di}/PTP_{oes} ratio (fig. 2b) as well as absolute values of PTP_{di} significantly decreased compared to SL, suggesting further recruitment of accessory respiratory muscles to assist impending failure of the diaphragm. These observations are indirectly confirmed by studies on hyperoxic exercise that showed a decrease of accessory muscle activation compared to normoxic exercise [24].

At HA, but not at SL, diaphragm recovery from fatigue took longer than 60 min (fig. 2a). It was associated with lower (three subjects) or equal (five subjects) external work at HA, with significantly reduced end-exercise PTP_{di} , and with blood lactate higher than baseline 60 min after exercise. As lactate clearance is delayed by hypoxia [30], and the increased level of circulating lactate and other metabolites has been implicated in the fatigue process [31], it might well be that hypobaric hypoxia and not increased workload, represents the major cause of the delay in recovery from exercise-induced diaphragm fatigue at HA.

In conclusion, exercise at high altitude challenges the respiratory muscles as a whole. In fact, not only can high altitude enhance diaphragm fatigue during exhaustive incremental exercise, but it also overloads accessory inspiratory muscles on account of hypobaric hypoxia alone, at least during acute exposure to the altitude tested. Whether this could affect exercise capability, particularly when ventilatory requirements become enormous (*e.g.* in acclimatized subjects at extreme altitudes), is still questionable. In fact, the effects of hypoxaemia are not confined to the respiratory muscles, since changes in oxygen supply affect locomotor muscles as well [24]. Further work is required to assess whether exercise-induced diaphragm dysfunction has any role in determining exercise limitation at high altitude.

Acknowledgements. The authors gratefully acknowledge the excellent work of E. Visetti in preparing the laboratory in the Rifugio Torino and assisting in the measurements performed there, and of M. Marucco, President of the "Soccorso Alpino Italiano" Regione Piemonte, Italy, who organized the teams staying at Monte Bianco. The authors also warmly thank L. Bucciardini, M. Carone, M. Linden, U. Zummo, and L. Spagnolatti for their very useful assistance. They are also indebted to C. Miscio, from Dept of Neurology for her help in performing studies with magnetic stimulation. Finally, the authors would also like to thank R. Allpress for her help in the preparation of this manuscript.

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