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ABSTRACT

The present investigation was intended to assess the consequences of an inspiratory load on the diaphragm central component of fatigue during exercise. We recorded the motor potential (MEP) evoked by transcranial magnetic stimulation of the motor cortex in ten subjects. The diaphragm and *rectus femoris* were studied before and 10, 20 and 40 min after two 16-min cycling exercise trials requiring 55% of $\dot{V}O_{2MAX}$: i) one with an inspiratory threshold load (E + ITL) corresponding to 10% of maximal inspiratory pressure and ii) the other without the load (E). Dyspnea, heart rate, electromyographic activity of the sternocleidomastoid, and diaphragm work were significantly higher in E + ITL than in E. Neither trial affected the response to phrenic magnetic stimulation, which was performed 15 and 25 minutes post-exercise, or the maximal inspiratory pressure (116 cmH_2O and 120 cmH_2O before E and E + ITL, respectively, and 110 cmH_2O and 114 cmH_2O at 30 minutes post-exercise). Whereas the amplitude of the diaphragm MEP was unaffected by E + ITL (+ 2.1 ± 29.4 %), a significant decrease was observed 10 minutes after E as compared to baseline (-37.1 ± 22.3 %) and as compared to E + ITL. The MEP amplitude of *rectus femoris* remained unchanged with E and E + ITL. The recruitment of synergistic agonists during E + ITL may have normalized the major ventilatory stress and reset up the excitability of the diaphragm pathway.

INTRODUCTION

Fatigue develops when striated skeletal muscles are confronted with loads that challenge their strength and endurance, involving both peripheral (downstream the neuromuscular junction) and central mechanisms (see 13 for a review). Central fatigue is common in humans (7, 8, 33) and is viewed by some as a mechanism to protect against exercise-induced muscle damage or exercise-induced homeostatic failure (13).

As with other skeletal muscles, inspiratory muscles are susceptible to fatigue both in response to specific loading (16) and after exhaustive endurance exercise. For example, highly fit normal volunteers exhibit decreased static inspiratory pressures after prolonged or extreme whole body exercise [*e.g.* a triathlon (17) or marathon (20)]. Because of the volitional nature of static inspiratory pressure manoeuvres, the question of the central or peripheral nature of the mechanisms involved has been left open. Johnson *et al.* (19) found convincing evidence of contractile alterations (peripheral fatigue) when they compared the results of bilateral supramaximal phrenic nerve stimulation before and after exercise-induced diaphragm fatigue in healthy humans. This does not, however, exclude a central component. Indeed, Verin *et al.* (35) demonstrated supraspinal diaphragm fatigue following incremental treadmill exercise. In this study, the depression of the diaphragmatie motor potentials evoked by transcranial magnetic stimulation was more pronounced and slower to recover than that of the quadriceps.

During CO_2-stimulated ventilation (38) or exercise, plastic changes in respiratory muscle recruitment occur. The contribution of the diaphragm tends to decrease, with the muscle acting increasingly as a flow generator rather than a pressure generator (2). The contribution of extradiaphragm inspiratory and expiratory muscles to ventilation augments so as to maintain the global efficacy of the ventilatory pump. This is true when ventilatory loading starts with fresh muscles (19) and is even more evident when the diaphragm has been previously fatigued (30). These adaptive strategies are central in nature and may have a

protective function for the diaphragm. The present study was designed to address a part of this issue in healthy volunteers. The specific aims were to determine i) whether moderate cycling exercise leads to a depression in motor evoked potentials and ii) how an imposed inspiratory threshold load [known to provoke a strong recruitment of extradiaphragm inspiratory muscles (39)] would influence this phenomenon. We hypothesized that the diaphragm responses to transcranial magnetic stimulation after exercise ewould be dependent on whether the exercise was performed with or without inspiratory threshold loading.

METHODS

Participants

Ten healthy volunteers (5 men, 5 women; age 21 to 33 yr; body mass index 18.7 to 25.8 kg.m^{-2}; all but one right-handed), free of any disease or significant medical history, were studied. Their maximal oxygen uptake ($\dot{V}O_{2MAX}$) ranged from 32.0 to 56.9 ml. min^{-1}.kg^{-1} (see **Table 1**). They were instructed to maintain their usual level of physical activity during the study period and not to modify their caffeine or nicotine consumption. They refrained from alcohol consumption, sedative intake, and heavy exercise during the 24 hours preceding the tests. Of note, an 11th subject who had been recruited dropped out of the study because he could not tolerate transcranial magnetic stimulation.

Table 1

The protocol had been approved by the appropriate ethics committee and all subjects gave their informed written consent for the study.

Preliminary evaluation

At the first visit, each subject underwent a physical examination and a 12-lead electrocardiogram (ECG). Maximal oxygen uptake was measured using an electronically braked cycle ergometer (Ergometrics 800, Ergoline, Germany) as follows. After an 8-minute

warm-up at a heart rate of about 110 beats per minute, the load was increased every minute by 25 watts until the subject was unable to continue despite vigorous coaching. During this procedure, the subjects breathed through a face mask (Hans Rudolph, Kansas City, MO, USA) connected to a pneumotachograph (Type 3, Hans Rudolph, Kansas City, MO, USA). Inspiratory and expiratory gas composition and flows were analyzed breath-by-breath using an automated system (Medisoft, Exp'air 1.26, Belgium). The metabolic and ventilatory parameters, including oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), expiratory flow ($\dot{V}E$), tidal volume (V_T), respiratory rate (RR) and duty cycle (ratio between inspiratory time and total time of the cycle), were monitored continuously and averaged over every 15-s period. Heart rate was recorded (Archimed) continuously by four ECG leads.

The following criteria had to be met to accept the highest $\dot{V}O_2$ mean over 15 s at the time of task failure as maximal: i) stabilization of $\dot{V}O_2$, ii) a respiratory exchange ratio >1.1, and iii) attainment of the age-predicted maximal heart rate (220-age).

The capacity of the subjects to produce static inspiratory pressures was measured in terms of the volitional maximal inspiratory pressure developed from residual volume against an occluded airway (PI_{MAX}). Maximal inspiratory pressure was determined as the highest pressure maintained over 1 s against an occlusion. Mouth pressure was measured by a differential pressure transducer (± 250 cmH_2O, Validyne, Northridge, CA, USA), digitized at 500 Hz (MP100 Manager V3.2.6, Biopac Systems, Inc., Santa Barbara, CA, USA) and stored on a computer.

Exercise protocol

Each subject performed two exercise (E) trials, one with an inspiratory threshold load (ITL) added (E + ITL) and one without (E). The runs were performed in random order, at a minimal interval of two days and at the same time of the day. Each trial consisted of a 5-

minute warm-up at 50% of the exercising load, immediately followed by a 16-minute cycling bout. The exercising load required 55% of $\dot{V}O_{2MAX}$ and was below the ventilatory anaerobic threshold determined according to Wasserman (37). Subjects were instructed to cycle between 60 and 70 rpm. Throughout the tests, gases were analyzed breath-by-breath using the automated system described above.

Inspiratory loading was achieved during the E + ITL runs by connecting an inspiratory threshold valve (threshold IMT, Respironics, Germany) to the inspiratory side of a two-way breathing valve (type 2700, Hans Rudolph, Kansas City, MO, USA) connected to the face mask. The valve was adjusted to open at an inspiratory pressure of about 10% of PI_{MAX}. The inspiratory load was added 1 minute after the beginning of exercise. Expiration remained free throughout testing. Mouth pressure and flow were continuously recorded during both trials from the set-up of the inspiratory load.

While exercising, the subjects were asked to rate their dyspnea every 90 s on a visual scale.

No instructions were given to the subjects regarding breathing pattern.

Muscle activity during exercise

The electromyographic (EMG) activity of the sternocleidomastoid muscles (SCM) and transdiaphragmatic pressures was monitored continuously during the two trials (E and E + ITL) on the six subjects numbered 5 to 10 in Table 1.

Spontaneous electromyographic activity of the SCM was obtained by surface recording using pairs of disposable silver cup electrodes. These were placed over the mid-point of the muscle belly (4). The raw EMG signals were full-wave rectified and integrated with time of burst to provide an integrated EMG activity index (EMG_i). During the 16-minute cycling bout, the subjects were asked to maintain their posture on the ergocycle and any part of the signal containing interference caused by head movement was removed.

The transdiaphragmatic pressure was determined as the difference between gastric and oesophageal pressures continuously recorded over the two exercise trials. The catheter tip pressure transducer (CTO-2, Gaeltec Ltd, Scotland, UK) was gently positioned in the oesophagus and stomach, after local anaesthesia of the nasal passage with xylocaine spray (xylocaine 5% Nebuliser, Astra Inc., France). Transdiaphragmatic pressure deflections were integrated over time (Pdi_i) and served to reflect the work of the diaphragm.

Transcranial magnetic stimulation

The responses to transcranial magnetic stimulation were studied in all subjects, who were seated comfortably in a quiet room, in a relaxed state.

Surface recordings of the diaphragm and *rectus femoris* electromyograms were obtained using pairs of disposable silver cup electrodes. The right costal diaphragmatic activity was recorded with active electrodes taped to the skin along the mid-clavicular line in the eighth intercostal space (34). For the *rectus femoris*, the electrodes were placed in the physiological axis over the belly of the muscle (25).

Transcranial magnetic stimulation was performed using a Magstim® 200 magnetic stimulator (The Magstim Company, Sheffield, UK) equipped with a conical butterfly coil (coil diameter 110 mm; maximal output 1.6 T). The optimal stimulation site and coil position were determined by testing several combinations at the lowest stimulation intensity evoking a response from both the *rectus femoris* and the diaphragm. To ensure constant body and coil positions, the shadow of the subject as projected from direct lateral lighting and the shadow of the coil in stimulation position were drawn on a board at the beginning of the first session and then carefully reproduced. Care was taken to perform transcranial magnetic stimulation with the diaphragm and the *rectus femoris* relaxed to avoid any modification in the response by facilitation, and at end-expiration (from visual inspection of rib cage movement).

A 10-minute rest period preceded the baseline measurements. At rest and 10, 20 and 40 minutes after the end of exercise, sets of five transcranial magnetic stimulations were performed at 100% of stimulator output. The time interval between stimulations was 30 s.

The electromyographic signals were digitized at 20,000 Hz for 260 ms after the stimulation (MP100 Manager V3.2.6, Biopac Systems, Inc., Santa Barbara, CA, USA), analyzed and then saved to a desktop computer. The MEP latency was measured as the time elapsed from the stimulus to the first departure of the electromyographic signal from baseline. Its amplitude was determined as the peak-to-peak difference of the first recognizable deflection.

Cervical magnetic stimulation and maximal inspiratory pressure

The six subjects in whom muscle activity was studied had a slightly modified exercise protocol: diaphragm responses to phrenic nerve stimulation were also recorded before exercise (after transcranial stimulations) and 15 and 25 minutes after exercise. The purpose of these measurements was to assess any peripheral fatigue that may have developed. These subjects also performed the PI_{MAX} manoeuvres described above (see *preliminary evaluation*) at rest and 30 minutes after the end of each exercise trial.

The phrenic nerves were bilaterally stimulated with cervical magnetic stimulation using 100% of the maximal output of a Magstim® 200 system, and a circular 90-mm coil (S/N, maximum output 2 T, pulse duration 0.05 ms; The Magstim Company, Sheffield, UK). Subjects were seated with the neck slightly flexed; they wore a noseclip and breathed through a mouthpiece with a small leak (to avoid glottis closure). Five stimulations were delivered at the functional residual capacity determined by visual control of chest movements, with at least 30 s intervals to avoid a twitch on twitch potentiation.

Mouth pressure measurement in response to phrenic nerve stimulation was performed as described in the *Preliminary evaluation* section. The amplitude of the twitch mouth pressure (twPmo) signal was measured as the difference between baseline and the peak pressure during

phrenic stimulation. The electromyographic (M-Wave) and mechanical responses to cervical magnetic stimulation were acquired similarly to the responses to transcranial magnetic stimulation (see above). We took particular care in verifying the absence of difference in the M-wave amplitude of the diaphragm.

Statistical procedures

The distributions of the amplitudes and latencies of the motor evoked potentials were checked for normality using the D test of Kolmogorov-Smirnov. Because none of the MEP distributions were normal, a log-transformation was applied. The normality of the distributions after transformation was confirmed by D values of 0.077 for the diaphragm in the E and E + ITL trials, and D values of 0.087 and 0.077 for the quadriceps in the E and E + ITL trials, respectively (all $p>0.2$). The latencies of the diaphragm motor evoked potentials were normally distributed during free and loaded exercise (all $D<0.1$ and $p>0.2$).

Two-way analysis of variance with repeated measures was used to determine differences in the mean values of MEP amplitudes and latencies over the duration of exercise and recovery (Statistica Kernel V 5.5 Statsoft, France). The analysis for MEP amplitudes was performed on log-transformed data.

When a main statistical effect was found by ANOVA, means were compared using a Newman-Keuls *post hoc* test.

Paired *t* tests were used to assess differences beween trials (with and without inspiratory load) when the effect of time was not analyzed.

Data are expressed as means ± SD unless otherwise stated. Differences were considered statistically significant when the probability *p* of a type I error was 0.05 or less.

RESULTS

Cardioventilatory data

Two subjects had to interrupt the loaded exercise trial before its scheduled end because of excessive hyperventilation inducing acute dizziness in one case and intolerable dyspnea in the other. As they stopped only 1 and 2 minutes before the end of exercise, respectively, they were maintained in the analyses.

Fig. 1

The mean minute ventilation, breathing frequency, tidal volume and duty cycle recorded over 15 minutes of the exercise were not different between the free and loaded runs (**Figure 1**), as reflected by p values of 0.94, 0.69, 0.13, and 0.27, respectively. However, the individual breathing patterns in response to the threshold load varied among the subjects. Overall, the mean inspiratory flow rate (V_T / Ti), which is assumed to reflect the ventilatory command, did not differ between the two runs.

Fig. 2

All the subjects but one scored a significantly higher dyspnea during the trial with inspiratory loading than during the free trial of whole-body exercise **(Figure 2A)**.

The mean heart rate was significantly higher during the loaded run than the free run (147 ± 15 *vs.* 144 ± 18 bpm, $p = 0.03$) **(Figure 2B)**.

Muscle activity

The sternocleidomastoid activity was significantly higher (5.3 ± 2.5 *vs.* 15.4 ± 10.4) when the inspiratory load was added (**Figure 3A, Figure 4**). Indeed, the clinical observation of rib cage dynamics supported these results.

Fig. 3

In the only three subjects whose diaphragm work could be measured during the two exercise runs, Pdi_i increased from 18.3 ± 6.1 cmH$_2$O.ms^{-1} in the free trial to 56.7 ± 9.0

Fig. 4

cmH$_2$O.ms^{-1} in the loaded one, and mean Pdi followed the same increment pattern (**Figure 3B, Figure 4**).

Cervical magnetic stimulation and maximal inspiratory pressure

The variance analysis showed that the maximal inspiratory pressure was not affected by the trial (exercise *vs.* loaded exercise), the effort (after *vs.* before, whether including an inspiratory load or not) or a combination of these factors (**Figure 5A**). Similarly, twitch mouth pressures did not change across conditions of time or exercise (**Figure 5B**). The M-wave amplitude also remained unaffected by these factors (Figure 6).

Responses to transcranial magnetic stimulation

ANOVA revealed an overall interaction effect between time of measurement and trial condition on the amplitudes of the diaphragm MEP (p = 0.038) The amplitudes were not significantly different between the two trials at rest (p=0.209). Ten minutes after the end of E, however, they were significantly lower than at baseline and at the same point in time after E + ITL (**Figure 7A**); 20 and 40 minutes after E, the amplitudes remained lower than at baseline, although not significantly so (p = 0.061 and 0.075, respectively). Conversely, E + ITL had no effect on the amplitudes of the diaphragm MEP (after *vs.* before, all p values >0.88).

The average diaphragm MEP latencies were 15.5 ± 1.1 ms at baseline in the free condition and 16.0 ± 1.1 ms in the loaded condition. The latencies were unchanged over time during a given session, and unaffected by ITL (p=0.77).

Figure 7B describes the evolution in the amplitudes of the *rectus femoris* motor evoked potentials with time. The MEP amplitudes were unchanged over time after E, and unaffected

by ITL. The MEP latencies also remained unchanged between and during the trials (p=0.98). The average latency at rest was 22.1 ± 2.5 ms for E and 22.4 ± 2.1 ms for E + ITL.

DISCUSSION

This study shows that non-exhaustive whole body exercise in normal individuals can induce neurophysiological changes compatible with supraspinal diaphragm fatigue, and that this effect is not present when the exercise is combined with a moderate level of inspiratory threshold loading.

Methodological issues

A decrease in the amplitude of the motor potentials evoked by transcranial magnetic stimulation can be interpreted in terms of supraspinal fatigue when no significant metrological changes occur. In the present study, we took particular care to maintain a constant body posture and a constant relationship between the stimulating coil and the scalp throughout the experiments (see Methods). In addition, because changes in the inter-electrode impedance or the spatial relationship between the electrodes and the recorded motor units could theoretically have played a role in the variations that we observed, we compared the amplitude of the responses to peripheral stimulation before and after exercise. We observed no variation in the electromyographic response to CMS, which was expected, given that this signal is generally unaffected by fatigue protocols involving non-isotonic efforts. We are thus confident that changes in the stimulus or changes in the recording conditions did not play a significant role in the phenomena that we observed.

The measurement of the pressure generated in response to CMS allowed for the control of peripheral muscle fatigue in this study. In terms of exercise intensity, ventilatory demand, and degree of loading, the experimental conditions were such that contractile fatigue was extremely unlikely to occur, and particularly so in the diaphragm (19). Indeed, the absence of variation in the twitch mouth pressure suggests that peripheral fatigue did not develop.

Finally, non-fatiguing efforts tend to potentiate the electromyographic response to transcranial magnetic stimulation (3). This "post-exercise facilitation" phenomenon is the result of a transient increase in the excitability of the alpha-motoneurons induced by muscular activity (28), a feature that we did not observe after either type of exercise run. The motor evoked potential latencies in our subjects, which were in the normal range for unfacilitated responses (29), did not differ from their baseline values after exercise. The maximal inspiratory manoeuvre has potential facilitatory effects but it was followed by 10 minutes of rest. The subjects remained quiet, *i.e.* motionless and silent, during the recovery. Post-exercise facilitation therefore does not seem likely to have been a confounding factor.

Central diaphragm fatigue in response to unloaded exercise

The twitch interpolation technique has been used to assess the degree of voluntary activation of a given muscle during a voluntary contraction (24, 21). It consists of superimposing supramaximal stimulations on the nerve commanding an already contracting muscle. When task failure is associated with an additional mechanical output in response to this stimulation, fatigue is assigned to a central component. Central diaphragmatic fatigue has been evidenced using this technique in various contexts (6, 21) characterised by diaphragm-oriented loading protocols. Verin *et al.* (35) seem to have been the first to provide arguments for central diaphragm fatigue after whole-body exercise. They found that both the diaphragm and the quadriceps exhibited post-exercise decrements in their responses to transcranial magnetic stimulation after an incremental walking exercise likely to require high ventilatory outputs. As compared to their study, we did not observe signs of supraspinal fatigue in the *recti femori*. The reason for this difference may lie in the nature of the exercise paradigm used: our protocol was specifically designed not to provoke exhaustion. The two studies are, however, consistent in that they suggest a greater susceptibility to supraspinal fatigue in the diaphragm than in the quadriceps. This result is compatible with the view that central fatigue has a protective function (13). It is also tempting to draw a parallel between the results of

these two studies and the fact that normal subjects achieve greater activation of the quadriceps than of the diaphragm during similar loading protocols (14). This feature has been called on by Guleria *et al*. (14) to explain the relative difficulty of experimentally producing diaphragm contractile fatigue in comparison with other muscles.

Of note, most works on supraspinal fatigue have involved intense muscular efforts, generally mono-articular and leading to task failure. The muscles under study have generally been small and distal (9, 28), with some exceptions (27, 33). This was not the case for the diaphragm in our experiments, which makes comparison with other results difficult. However, supraspinal fatigue has been observed in several muscles as a result of whole body exercise (18) (as opposed to specific limb solicitation).

Effects of inspiratory threshold loading on the post-exercise diaphragm response

Increased ventilatory demand is often associated with changes in the activity and recruitment pattern of the respiratory muscle groups (19, 26). These changes generally tend to put the burden of the ventilatory load on extradiaphragm muscles. For example, exercise is normally associated with a precocious and strong recruitment of abdominal muscles that directly contribute to the ventilatory work by their expiratory action. They also indirectly help inspiration by bringing the rib cage at end-expiration below its equilibrium position, therefore making rib cage elastic recoil available for inspiration (10). Rib cage and neck muscles are also recruited during exercise. They contribute directly to the expansion of the rib cage, and decrease rib cage distortability (21). As a result, it has been shown that the nature of the diaphragm contraction changes during exercise (2) and therefore its energy expenditure is not increased disproportionately. Studies, particularly on limb muscles, have clearly demonstrated that a given task can be accomplished with different recruitment strategies. For example, fatigue of the *vastus lateralis* has been shown to modify the recruitment of synergistic muscles, thereby preserving the success of a knee extension task (1). Given the compensatory

mechanisms that are developed in patients with diaphragm paralysis (15) and in healthy subjects exercising with fatigued inspiratory muscles (30), it also appears reasonable to assume that changes in the pattern of extradiaphragm respiratory muscle activity would allow the ventilatory demand to be met in spite of reduced diaphragm activity. To support this, Johnson *et al.* (19) showed that twitch transdiaphragmatic pressure was reduced at all lung volumes after task failure in 12 healthy volunteers with a variety of fitness levels exercising to exhaustion at 85 and 95% of their maximum oxygen uptake. In this study, the contribution of the diaphragm to the respiratory motor output tended to decrease with the duration of the effort, with a shift of the load toward other muscles.

Inspiratory loading favours the recruitment of inspiratory neck and rib cage muscles (30), and the amplitude partly determines endurance. For this study, the choice of 10% of the maximal inspiratory pressure was based on preliminary studies that showed the load to be demanding and capable of generating strong neck and rib cage muscle contractions. The literature (5, 35) reveals that this load is associated with a normal respiratory duty cycle and is unlikely to induce low frequency diaphragm fatigue, which had to be avoided in this study. The recruitment of additional muscles with loading is observed in patients with chronic obstructive pulmonary disease and in healthy subjects. Both resistive (16) and threshold (11) loading lead to strong solicitation and sometimes fatigue (16, 39) of extradiaphragmatic muscles. The ITL added to the burden of the exercise-related increase in ventilatory demand consistently resulted in recruitment of extradiaphragmatic muscles, as shown by the electromyographic recording of the sternocleidomastoid muscles. Moreover, our subjects reported dyspnea during the loaded protocol, whereas this was not the case during the "free" protocol. Yet Fitting *et al.* (12) and Ward *et al.* (36) both clearly showed that recruiting the sternocleidomastoid and parasternal intercostal muscles rather than the diaphragm to perform a given respiratory task is strongly associated with dyspneic sensations. Therefore, we suggest

that the redistribution in the respective roles of the inspiratory muscles is related to the disappearance of the post-exercise depression in diaphragm MEPs in the E + ITL condition.

This is not a simple assertion that the decrease in motor evoked potentials is due to lower recruitment of the diaphragm. There is probably no correlation between the amount of work produced by a muscle group at a given time and the fatigue of its command. Instead, we propose that the decreased excitability after exercise is linked to the reorganization of the diaphragm's motor command. When moderate exercise is not prolonged, this reorganization could optimize the use of resources. Changing the breathing has already been presented as an optimization strategy that is implemented during the development of fatigue (11). Consequently, it could occur without any change in the mechanical output or alteration in the maximal performance (PI_{MAX}, in this case), but it may still be a limiting factor when exercise is prolonged, probably through the development of dyspnea (31, 22). Sufficient data are lacking to venture a hypothesis about whether the adaptation depresses the excitability of the motor command – as reflected by a decrease in MEP amplitude - or whether decreased excitability results in reorganized recruitment. We nevertheless suspect that the second position will be borne out and propose that early in the E + ITL trial, mechanical afferences led to a relative inhibition of the diaphragm motor command that later normalized. Even though it results in dyspnea, which is another constraint, the recruitment of neck and rib cage muscles controls the major stress to the respiratory system, *i.e.* the inspiratory load. It is possible that in this condition the excitability of the respiratory pathways is reset up during the exercise itself and is not influenced by slighter changes in the homeostasis. Longer inspiratory loaded exercise would be interesting to study to assess whether dyspnea continues to act as a safety feature by leading to the end of exercise before the respiratory system adapts to metabolic changes through variation in the excitability of its command. In contrast, a finer regulation of the diaphragm command, based on both mechanical and chemical afferences,

could be sought during exercise.This would result in a prolonged relative domination of inhibitory over excitatory mechanisms.

In conclusion and in spite of its limitations, this study seems to contribute to the current body of knowledge suggesting that several mechanisms act in concert to protect the diaphragm against overload-induced damage. These mechanisms include a reduced central activation in loaded conditions (6), the early occurrence of supraspinal fatigue or inhibition (21), and agonist recruitment to overcome an increased demand.

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LEGENDS

Table 1. Individual characteristics of the subjects. MAP: maximal aerobic power, defined as the highest power the subject was able to maintain for one minute during the incremental test. In the gender section, m stands for male and f for female.

Figure 1. Individual and mean variations between the two sessions: exercise (E) and inspiratory-loaded exercise (E + ITL), for the following ventilatory parameters: A) ventilatory output, B) breathing frequency, C) tidal volume, D) respiratory duty cycle, *i.e.* ratio between inspiratory time and total time of a cycle. NS: non-significant.

Figure 2. Individual and mean ($\pm$ SD) variations between the two sessions: exercise (E) and inspiratory-loaded exercise (E + ITL), for A) dyspnea rated on a Borg scale and B) mean heart rate. * : $p<0.05$; **: $p<0.01$.

Figure 3. A) Mean and SD integrated electromyographic activity (EMGi) of the sternocleidomastoid muscles over the time of E ($\square$) and E + ITL ($\blacksquare$). n = 6. **: $p<0.01$. B) Mean and SD amplitude of the average deflection of Pdi during the sessions; no statistical analysis was performed on these data since the number of subjects is small (n = 3).

Figure 4. Representative pressure traces recorded in one subject at the beginning of E (A) and during a corresponding period in E + ITL (B). The transdiaphragmatic pressure is in grey, gastric pressure is represented by the black trace in phase with Pdi, and oesophageal pressure is the remaining black trace. The electromyographic activity of the sternocleidomastoid is displayed below the pressure traces.
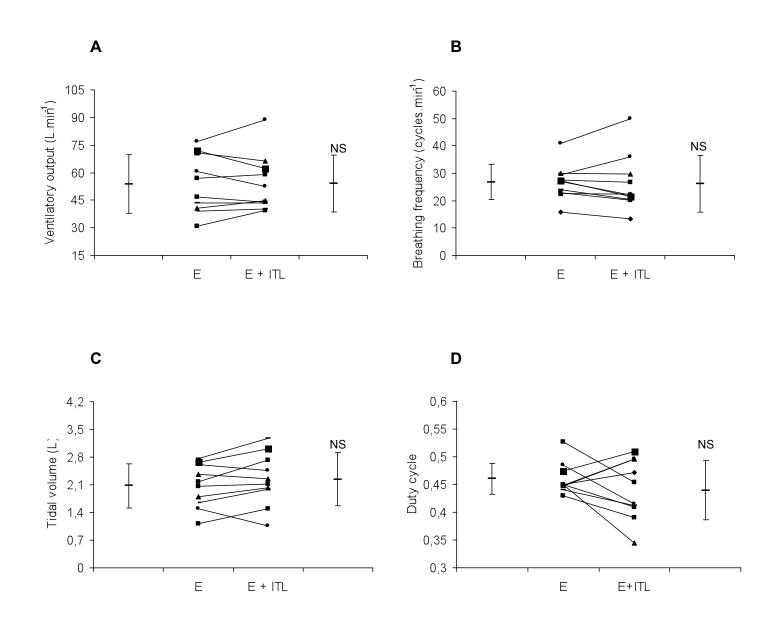
Figure 5. A) Mean and SEM for PI_{MAX} before (rest) and 30 minutes (P30) after the end of exercise without ($\square$ = exercise trial) and with ($\blacksquare$ = inspiratory loaded exercise trial) an inspiratory load. B) Mean and SEM for mouth pressure in response to cervical magnetic stimulation before (rest), and 15 (P15) and 25 minutes (P25) after the end of the trials. n = 6.
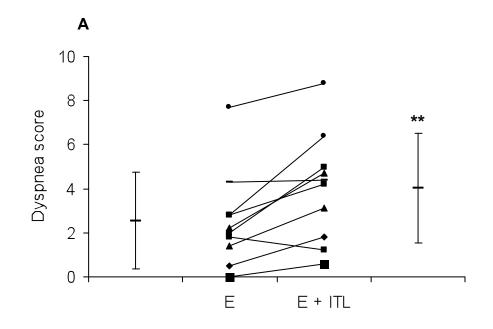
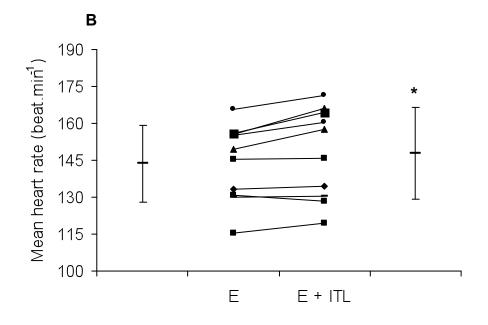The interaction effect was non-significant (NS, p = 0.96 and 0.71 in A and B, respectively).

Figure 6. Representative M-wave trace recorded in one subject at the measurement time points of baseline and 15 and 25 minutes post-exercise (post 15 min and post 25 min, respectively) of the exercise trials (A) and the inspiratory loaded exercise trials (B).
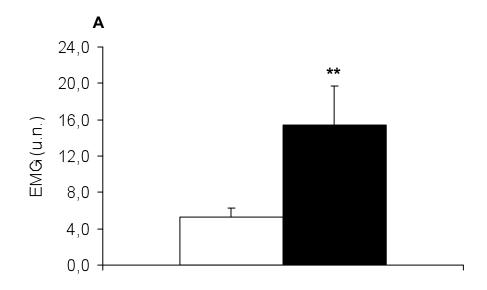
Figure 7. Mean and SEM for MEP amplitudes of the diaphragm (A) and *rectus femoris* (B) at rest, and 10 (P10), 20 (P20) and 40 (P40) minutes after the end of exercise. $\square$ exercise (no inspiratory load), $\blacksquare$ exercise with inspiratory load. * : $p<0.05$ as compared to rest in the same condition; # : $p<0.05$ as compared to the other condition at the same time. NS: non-significant interaction effect between the time and condition effects.
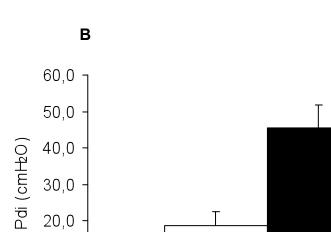
TABLE 1

Subject	Gender m / f	Age (years)	Height (cm)	Weight (kg)	MAP (W)	$\dot{V}O_{2MAX}$ $(ml^{-1}.min^{-1}.kg^{-1})$	PI_{MAX} (cmH_2O)
1	f	30	168	65	225	38.0	135
2	m	30	175	79	350	56.9	146
3	f	33	169	59	175	36.0	105
4	m	24	184	81	300	43.5	202
5	m	25	182	62	290	56.3	175
6	m	27	180	77	350	52.0	159
7	f	22	170	55	200	36.9	100
8	f	22	176	66	275	35.4	135
9	f	26	163	60	200	32.0	112
10	m	21	185	79	350	46.6	132
Mean		**26**	**175**	**68**	**276**	**43.4**	**140**
SD		4	7	9	66	9.1	32

FIGURE 1

FIGURE 2

FIGURE 3

A

EMG (u.n.)

**

B

Pdi (cmH2O)

FIGURE 4

A

B

0 ⌡10 cmH$_2$O

1s

0 ⌡0.1 mV

FIGURE 5

FIGURE 6

FIGURE 7