

Increased ventilation does not impair maximal voluntary contractions of the elbow flexors

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Running head: Elbow flexor force unaffected by increased ventilation

Abstract

Exercise performance is impaired by increased respiratory work, yet the mechanism for this is unclear. This experiment assessed whether neural drive to an exercising muscle was affected by cortically-driven increases in ventilation. On each of five days, eight subjects completed a 2 min maximal voluntary contraction (MVC) of the elbow flexor muscles, followed by 4 min of recovery, while transcranial magnetic stimulation tested for suboptimal neural drive to the muscle. On one day, subjects breathed without instructions under normocapnia. During the 2-min MVC ventilation was ~3.5 times that at rest. On another day, subjects breathed without instruction under hypercapnia. During the 2-min MVC, ventilation was ~1.5 times that on the normocapnic day. On another two days under normocapnia, subjects voluntarily matched their breathing to the uninstructed breathing under normocapnia and hypercapnia using target feedback of the rate and inspiratory volume. On a fifth day under normocapnia, the volume feedback was set to each subject's vital capacity. On this day, ventilation during the 2-min MVC was ~ twice that on the uninstructed normocapnic day (or ~ 7 times rest). The experimental manipulations succeeded in producing voluntary and involuntary hyperpnea. However, maximal voluntary force, fatigue and voluntary activation of the elbow flexor muscles were unaffected by cortically- or chemically-driven increases in ventilation. Results suggest that any effects of increased respiratory work on limb exercise performance are not due to a failure to drive both muscle groups optimally.

Keywords: voluntary activation; voluntary hyperventilation; maximal contraction; respiratory muscles

Introduction

When two motor tasks are carried out simultaneously, performance of one or both tasks is often impaired. This is particularly common when the tasks involve significant effort. For example, if a subject performs a reaction time task with one hand and a steady contraction with the other, reaction times are slower and more errors are made if the contractions are stronger (27).

Performance is further impaired with fatigue (27). Even when the tasks are cognitively simple but involve high effort, like maximal isometric contractions of two muscle groups, deficits in performance are often, but not always, reported (e.g. 23, 26, 34, 48). Although biomechanical constraints are sometimes a problem, force production can be limited by the ability of subjects to direct neural drive to both muscle groups simultaneously. When there are no deficits in the maximal force generated by either muscle group, the circumstances usually involve well-practised combinations of contractions such as bilateral leg extensions in subjects who do weight training (e.g. 24).

In some circumstances, there are interactions between limb exercise and respiration. Exercise performance and limb muscle contractile function can be impaired by a period of increased respiratory work, such as voluntary hyperventilation (30, 52) (though for no difference see 11, 40, 49), breathing CO₂ mixtures (3, 4, 6), and increased inspiratory or expiratory resistance (22, 25, 29, 35, 38, 47). Conversely, task performance benefits when the work of breathing is decreased by continuous positive airway pressure or proportional assist ventilation (13, 22, 33, 35), or by breathing hyperoxic or HeO₂ mixtures (3, 5, 13). The adverse effects on performance occur with high levels of respiratory work which fatigue the inspiratory muscles. Blood flow to the exercising limb muscles is reduced by sympathetic nerve activity thereby limiting exercise

performance (8, 10, 20, 21, 37). This reflex response to fatigue of the diaphragm may allow it extra blood flow. The diaphragm is estimated to require up to 16% of the total cardiac output during heavy exercise (1, 20, 21). In addition, this increased peripheral fatigue is likely to increase firing of fatigue-sensitive muscle afferents which may feed back to the CNS to reduce neural drive to the muscles (3)

It is not clear whether other neural constraints might apply when a task involving limb muscles is performed in conjunction with a task involving the respiratory muscles. The respiratory muscles are unique in that they are driven automatically from brainstem centres, but can also be driven voluntarily from the cortex. If one neural limit to simultaneous tasks lies in an inability to generate sufficient descending drive from the cortex (e.g. 23, 26, 34, 48), then voluntary hyperpnea might impair neural drive to limb muscles more than chemically-driven hyperpnea. Voluntary activation of a muscle can be measured by twitch interpolation methods: stimulation of the motor nerve or motor cortex can elicit an increment in the force generated in active muscles, even during maximal voluntary efforts (14 for review). An increment in force evoked by stimulation of the motor cortex implies that the motor cortex is not fully recruited in a maximal effort (43, 45). An increase in the force increment, or superimposed twitch, over the course of sustained exercise suggests a progressive decline in voluntary activation and is the marker of supraspinal fatigue (15, 43).

In this study, we measured whether increased respiratory muscle work had an impact on voluntary activation during maximal voluntary contractions of the elbow flexors, and in particular whether this differed when the respiratory activity was driven from the cortex (voluntary

hyperpnea) or from the brainstem by reflex-mediated chemical drive. Typically, voluntary activation falls progressively during a sustained maximal contraction. Subjects show central fatigue and are unable to maintain their initial level of drive to the muscle (15). Thus, the performance of a sustained maximal contraction should be sensitive to any added impairments of drive related to simultaneous performance of respiratory tasks. Our hypothesis was that high levels of voluntary ventilation would impair subjects' ability to drive the elbow flexor muscles maximally during a fatiguing contraction.

Methods

Participants

Eight healthy adult subjects (aged 23-47 years, 5 women; 1.63-1.89 m; 61-85 kg) completed the experiment. Subjects varied from sedentary to exercising 4 times/week. All subjects gave their written informed consent, and all experimental procedures were approved by the local ethics committee and were conducted according to the Declaration of Helsinki.

Set up

Subjects sat with the right arm flexed to 90° in an isometric myograph that measured flexion torque at the elbow (transducer linear to 2kN, X-tran, Melbourne Australia; (2)). The forearm was vertical and supinated, and strapped to the myograph just proximal to the wrist. Feedback of flexion torque was provided to the subject by an LED display.

Subjects were connected via a mouthpiece to a partial rebreathing circuit for controlling end-tidal CO₂. A reservoir bag was placed in the circuit, as well as a manually operated valve which

allowed fresh (room) air to enter the circuit, and which was controlled by an experimenter monitoring the end-tidal CO₂ (Datex Normocap CO₂ monitor, Helsinki, Finland). Oxygen saturation was monitored with a pulse oximetry probe (Ohmeda Biox 3740 pulse oximeter, Louisville, CO, USA) on the left middle finger, and remained >95% throughout in all subjects. A second LED display provided target and feedback information on ventilation. The mouthpiece was connected via a two-way valve with the inspiratory port connected to a pneumotachometer (Hans Rudolph, Kansas City, MO, USA). The flow signal was integrated to give inspired volume.

Surface electromyograms (EMG) were recorded with electrodes (Ag/AgCl, 10 mm diameter) fixed to the skin (cleaned with alcohol and mild abrasion) overlying the muscle bellies of biceps brachii, brachioradialis and triceps brachii. Surface signals were amplified (300-1000 times) and filtered (16-1000 Hz; CED 1902 amplifiers, Cambridge Electronic Design, Cambridge, UK). Force and EMG signals were sampled at 2000 Hz through a laboratory interface for offline analysis (CED 1401 interface, Spike 2 software, Cambridge Electronic Design, Cambridge, UK).

Brachial plexus stimulation

Single electrical stimuli were delivered to the brachial plexus via a cathode in the supraclavicular fossa (Erb's point) and an anode on the acromion (100 μ s duration, constant current, DS7AH, Digitimer, Welwyn Garden City, UK). In each experimental session, stimulus intensity was gradually increased until no further increase was observed in the resting compound muscle action potential (M-wave) of biceps brachii, brachioradialis and triceps brachii muscles. Stimulus intensity was set at 50% above this level during the experiment, and ranged between 75 and 240

mA. The average amplitude of the resting maximal M wave (M_{max}) was 14.2 ± 5.2 mV for biceps, 8.0 ± 3.7 mV for brachioradialis and 4.4 ± 1.6 mV for triceps.

Motor cortical stimulation

Transcranial magnetic stimulation (Magstim 200, Magstim Co., Dyfed, UK) was used to stimulate the motor cortex. A circular coil (13.5 cm outside diameter) positioned over the vertex and oriented to preferentially activate the left motor cortex elicited motor evoked potentials (MEPs) in biceps brachii, brachioradialis and triceps brachii muscles. Stimulator output (47-75% of maximum) was set during brief maximal voluntary contractions (MVCs) to obtain a large MEP in the biceps brachii ($> 60\%$ of M_{max}) and a small MEP in the triceps ($< 20\%$ of M_{max})(45) Stimulus intensity was set in the first experimental session for each subject and remained constant throughout the study.

Experimental protocol

Subjects completed similar experimental protocols on 5 separate days at least 3 days apart. On each day, subjects initially performed 6 sets of 3 brief contractions. Sets comprised a brief (2-3 s) MVC of the elbow flexor muscles, with motor cortical and brachial plexus stimulation delivered during the MVC (see Figure 1A), followed at 8-s intervals by contractions to 75% and 50% MVC, with motor cortical stimulation during each contraction (these sub-maximal contractions were used to obtain an estimate of the resting twitch, see below). During the first 3 control sets of contractions, subjects breathed room air, while for the second 3 sets subjects breathed through the mouthpiece according to their assigned ventilation protocol (see below). Sets of contractions were separated by intervals of at least 1 minute to minimise fatigue. Subjects then performed a 2-

min MVC, with motor cortical and brachial plexus stimulation delivered 5 seconds apart, every 20 seconds. Brief MVCs with motor cortical and brachial plexus stimulation were performed at 30s, 1, 2, 3 and 4 min after the end of the 2-min MVC. Each of these MVCs was followed by contractions to 75% and 50% MVC with cortical stimulation. During the fatiguing contraction and the recovery period, subjects continued to breathe through the mouthpiece according to their assigned ventilation protocol.

Subjects followed different ventilation protocols on each of the 5 days. On Day 1 (“Control”), subjects were allowed to breathe normally (i.e., with no breathing instructions, other than not to hold their breath), and the valve on the breathing circuit was fully open to allow fresh room air to enter. CO₂ was not experimentally manipulated (see Figure 1B).

On the second day (“Matched Control”), subjects matched their breathing to that from the first day. Targets of inspiratory volume, inspiratory time and expiratory time were provided through an LED display which showed volume as increasing lights during inspiration. Three separate targets were set during the experiment. These were derived from each subject’s mean data measured separately during the control period, the 2-min MVC and the recovery period on the Control day. CO₂ was maintained at levels similarly calculated for each period. Thus, mimicking the Control day, CO₂ was allowed to fall during the 2-min MVC.

On the third day (“CO₂-driven Hyperpnea”), subjects again breathed under no instructions, but end-tidal CO₂ was maintained at a high level. This level (41-54 mmHg) was set for each subject by allowing end-tidal CO₂ to rise by rebreathing until ventilation was increased to 2-3 times their

ventilation during the Day 1 control period. The same level of end-tidal CO₂ was maintained throughout the control MVCs, 2-min MVC and recovery.

On the fourth day (“Voluntary Hyperpnea”), subjects matched their breathing to the CO₂-driven Hyperpnea day with targets provided for inspiratory volume, inspiratory time and expiratory time, but with end-tidal CO₂ matched to Control day levels. Ventilation and CO₂ were separately matched for control MVCs, 2-min MVC and during recovery.

On the fifth day (“Maximal Voluntary Hyperpnea”), subjects matched their breathing to feedback which used their vital capacity as the target inspiratory volume and the inspiratory and expiratory times derived from CO₂-driven Hyperpnea. End-tidal CO₂ was matched to Control day levels. Subjects were instructed that if they could not match this feedback, they were to focus on achieving the tidal volume as fast as possible, rather than matching the rate.

Data extraction

Mean elbow flexion torque was calculated over 200 ms prior to each motor cortical stimulus and normalized to the off-mouthpiece control MVCs on each day. Increments in force evoked by motor cortical stimulation during all contractions were measured and normalized as a percentage of the ongoing MVC force. Voluntary activation during the brief control MVCs was calculated using the formula: Voluntary activation = (1-(superimposed twitch/estimated resting twitch)) X 100. The y-intercept of a linear regression between superimposed twitch amplitude and voluntary force for each set of 3 contractions (50%, 75% and 100% MVC) was used to estimate the resting twitch (45, 46). An estimated resting twitch was used because motor cortical neurones and

motoneurons are more excitable during voluntary contraction. Thus, cortical stimulation during rest does not activate the same motor units as cortical stimulation during contraction and does not produce a comparable twitch (45). For each muscle, the size of motor evoked potentials (MEPs) following motor cortical stimulation and the maximal M-wave (M_{max}) evoked by brachial plexus stimulation were measured as the area under the curve between set cursors which encompassed each potential. The area of the MEP in each muscle was normalized to the area of M_{max} elicited closest in time. Root mean square EMG amplitude (rms EMG) was measured over 200 ms before cortical stimuli for MVCs. For each muscle and on each day, rms EMG was expressed as a percentage of the control MVCs for that day. The duration of the silent period following cortical stimulation was measured as the time from the stimulus to the resumption of voluntary EMG. For each subject, the difference in silent period duration from that measured during the control contractions on each day was calculated.

Measurements of ventilation and end-tidal CO_2 were made at rest between control contractions and in the recovery period (the last 5-6 breaths before each brief MVC), over the first 4-5 breaths of the 2-min MVC and then for 4-5 breaths before each motor cortex stimulus. All minute ventilation measures were normalized to the control period on the Control day.

Statistical analysis

Minute ventilation measures were entered into a repeated measures two-way ANOVA with the within-subjects factors *Day* and *Time*. Only main effects are reported here. Separate analyses considered the 2-min MVC and the recovery periods, with polynomial contrasts on the Time factor (although only linear and quadratic trends are considered in this paper). Four single degree

of freedom planned contrasts on the Day factor were performed. To assess whether subjects adequately matched their target feedback, the Control day was compared with the Matched Control day, and the CO₂-driven Hyperpnea day (Day 3) was compared with the Voluntary Hyperpnea day (Day 4). To assess whether ventilation was significantly increased under the hyperpnea conditions, the mean of the control days was compared with the mean of the hyperpnea days (Days 3 and 4). Lastly, to assess whether ventilation was further increased under the maximal ventilation condition, CO₂-driven Hyperpnea was compared with Maximal Voluntary Hyperpnea.

Similarly, MVC force, superimposed twitch and the EMG-derived measures were also subjected to the same two-way ANOVAs and contrasts, with only main effects reported. Additionally, a two-way ANOVA was performed comparing the values off and on the mouthpiece during the control periods. Identical contrasts were performed on the Day factor, to assess whether the changes in breathing significantly affected MVC performance.

CO₂ was also entered into a two-way (Day x Time) ANOVA, with linear and quadratic trends on the Time factor. However, a different approach to the Day factor was used: as the end-tidal CO₂ was expected to be the same except for during the CO₂-driven Hyperpnea, repeated contrasts were used. That is, each normocapnic day was compared to the subsequent normocapnic day (the Control day was compared to the Matched Control day, Matched Control to the Voluntary Hyperpnea, and the Voluntary Hyperpnea to the Maximal Voluntary Hyperpnea day), and the CO₂-driven Hyperpnea day was compared to the Control day.

All contrasts have (1,7) degrees of freedom. As the contrasts were planned and there were no more of them than the degrees of freedom for effect, no Bonferroni-type adjustment to alpha was necessary (41).

Results

We first consider the success of our experimental manipulations on producing different levels of voluntary and involuntary ventilation, then we consider the effect this had on performance of the 2-min MVC of the elbow flexors and associated EMG measures.

Minute Ventilation

Minute ventilation increased to ~3.5 times control levels as soon as the 2-min MVC began (see Figure 2A; Table 1 & 2), mainly due to a rise in breathing rate, but showed no further reliable increase over the sustained contraction (linear $F < 1$, $p = 0.631$). Minute ventilation was well-matched between the Control day and the Matched Control day ($F < 1$, $p = 0.856$). The increase from rest to during the sustained MVC was from 12.6 ± 3.0 l to 45.5 ± 12.2 l (mean \pm SD) on the Control day and from 12.6 ± 2.3 l to 46.3 ± 13.2 l on the Matched Control day. Ventilation was significantly increased for hyperpnea days compared to control days (~1.5 times during the 2-min MVC), mainly due to an increase in inspiratory volume ($F = 15.8$, $p = 0.005$) but was lower for the Voluntary Hyperpnea compared to CO₂-driven Hyperpnea ($F = 14.2$, $p = 0.007$), due to the failure of subjects to reach the target volume during Voluntary Hyperpnea. With CO₂-driven Hyperpnea, minute ventilation went from 27.3 ± 7.0 l at rest to 64.8 ± 13.3 l during the 2-min MVC whereas with Voluntary Hyperpnea it went from 25.4 ± 7.0 l to 57.6 ± 11.2 l. As intended, ventilation was further increased for Maximal Voluntary Hyperpnea (55.6 ± 14.5 l at

rest and 87.6 ± 13.0 l during the 2-min MVC) compared to CO₂-driven Hyperpnea ($F = 12.9$, $p = 0.009$), with a slightly slower rate ($88 \pm 12\%$) but much higher tidal volume ($158 \pm 34\%$). Hence, during Maximal Voluntary Hyperpnea, ventilation during the 2-min MVC was approximately double that on the control day. That is, ~ 7 times normal breathing at rest.

Ventilation declined towards control levels with the end of the sustained contraction, and then remained stable at approximately the level of the control periods (linear $F = 14.0$, $p = 0.007$; quadratic $F = 12.1$, $p = 0.010$). Because constant target levels were set for the duration of the recovery period, matching of ventilation was poor initially (Control vs. Matched Control: $F = 15.3$, $p = 0.006$; CO₂-driven Hyperpnea vs. Voluntary Hyperpnea: $F = 101.9$, $p = 0.000$). However, ventilation was increased for the hyperpnea compared to control days ($F = 24.8$, $p = 0.002$), and further increased for Maximal Voluntary Hyperpnea ($F = 27.1$, $p = 0.001$).

CO₂

In order to assess the chemical drive leading to hyperpnea, it was necessary to consider CO₂ levels during the experiment. These are displayed in Figure 2B. Although end-tidal CO₂ dropped as soon as the 2-min MVC began, there were no significant changes in expired-CO₂ levels over the 2-min contraction (linear and quadratic F both < 1). As intended, expired CO₂ levels were significantly increased for CO₂-driven Hyperpnea compared to the Control day ($F = 157.1$, $p = 0.000$), but there were no significant differences between the normocapnic days (all $p > 0.05$).

A similar pattern of results was observed in recovery. There were no reliable changes in CO₂ over time (linear and quadratic F both < 1). As planned, CO₂ levels were significantly higher on

the CO₂-driven Hyperpnea day ($F = 95.6$, $p = 0.000$), and were not significantly different between the normocapnic days (all $p > 0.05$).

MVC force

MVC force (as a percentage of control MVCs on each day) is displayed in Figure 3A. There were no significant differences between MVCs performed off and on the mouthpiece ($F = 3.9$, $p = 0.089$), and this result was the same over all days (all $p > 0.101$). MVC force decreased quickly over the first minute and then more slowly in the second minute of the sustained MVC (linear $F = 526.1$, $p = 0.000$; quadratic $F = 164.839$, $p = 0.000$). There were no differences between days (all $p > 0.170$).

During recovery, the MVC force increased steadily (linear $F = 80.8$, $p = 0.000$) and in a similar way on most days, although recovery forces were significantly higher on the Matched Control day compared to the Control day ($F = 6.9$, $p = 0.034$).

Superimposed twitches

The amplitude of the superimposed twitches evoked by cortical stimulation during MVCs throughout the experimental protocol are displayed in Figure 3B for the group and in Figure 4 for a single subject for days 1 and 5 (where ventilation was most disparate). There were no differences in the superimposed twitches or voluntary activation during brief control elbow flexor MVCs when subjects were breathing with or without a mouthpiece. Overall, voluntary activation in the control MVCs during normal breathing was $91 \pm 2.9\%$ (mean \pm SD) and when breathing through the mouthpiece as dictated by the various protocols, $91 \pm 3.0\%$. The superimposed twitch

increased over the sustained maximal contraction (linear $F = 29.9$, $p = 0.001$; quadratic $F = 30.3$, $p = 0.001$). There were no significant main effects for the different days (all $p > 0.336$).

The superimposed twitch recovered almost to baseline levels within 30 s of the end of the 2-min MVC, and continued to decline thereafter (linear $F = 9.9$, $p = 0.016$). No main effects for the Days factor reached significance (all $p > 0.116$).

EMG measures

The EMG measures assessed during the protocols changed as expected during the 2-min MVC and recovery period (42, 44). Some isolated statistically significant differences between breathing conditions were found for some measures.

The amplitude of rms EMG in biceps and brachioradialis decreased during the sustained MVCs and increased in the recovery period. Two comparisons between days of rms EMG during the 2-min MVC were significant for individual muscles (Control > Matched Control for biceps, $F = 15.6$, $p = 0.006$; CO₂-driven Hyperpnea > Voluntary Hyperpnea in brachioradialis, $F = 6.9$, $p = 0.034$). As these differences each occurred in only one of the elbow flexors and did not result in changes in force, their physiological significance is doubtful.

MEPs in biceps and brachioradialis showed no significant differences between the 5 breathing protocols. Both Mmax and the MEP increased during the 2-min MVC (all linear and quadratic $p < 0.05$). At the end of the sustained contraction the area of Mmax in biceps and brachioradialis averaged $156 \pm 17\%$ and $142 \pm 22\%$ of control values respectively. However, the increase in the MEP (biceps to $178 \pm 42\%$ and brachioradialis to $172 \pm 40\%$ of control) was greater, such that

when the MEP was normalized to Mmax elicited close in time, the MEP still grew significantly during the sustained effort (biceps, quadratic $F = 8.5$, $p = 0.022$; brachioradialis, quadratic $F = 13.0$, $p = 0.009$). The MEPs in both muscles recovered to baseline levels within 30 s of the end of the sustained contraction. The silent period lengthened during the 2-min contraction by an average of 47 ± 30 ms in biceps (linear $F = 7.8$, $p = 0.027$; quadratic $F = 6.0$, $p = 0.044$) and 54 ± 33 ms in brachioradialis ($F = 1.4$, $p > 0.05$), and recovered quickly in both muscles. The increase in duration of the silent period in biceps but not brachioradialis was greater on the Control than Matched Control day ($F = 7.3$, $p = 0.031$). There were no other significant effects of Day.

Discussion

This study shows that performance of a 2-min sustained isometric maximal contraction of the elbow flexors is not affected by increased voluntary activity of the respiratory muscles. In each experiment, during the 2-min sustained MVC of the elbow flexors voluntary force fell, indicating the development of fatigue. At the same time the superimposed twitch evoked by cortical stimulation increased in amplitude, indicating a progressive failure of voluntary activation that can be attributed to suboptimal output from the motor cortex (15, 45). By definition, the fall in voluntary activation demonstrates the development of central fatigue (14). However, neither voluntary force nor voluntary activation of the elbow flexors was further reduced when subjects made high respiratory efforts. Peripheral and central fatigue developed in a similar way whether subjects breathed normally or hyperventilated, and whether subjects were normocapnic or hypercapnic. Our results indicate that high levels of voluntary or involuntary drive to the respiratory muscles do not impair drive to the limb muscles. This is in contradiction to our hypothesis that high levels of voluntary drive to the respiratory muscles would impair arm muscle performance because subjects would be unable to direct sufficient motor cortical drive to both

muscle groups simultaneously, as has been described with bilateral contractions of limb muscles (26, 34, 48).

Subjects performed four different ventilation protocols to test various possible mechanisms which might have impaired limb muscle performance compared to performance when breathing was not controlled. First, subjects were required to use visual feedback to follow targets of respiratory volume and timing. Here, we expected that attention would be required to follow the feedback and the origin of the neural drive to the respiratory muscles would be altered from largely automatic to voluntary, and thus would require additional output from the motor cortex (9, 12, 28, 31). Second, subjects were exposed to high CO₂ but given no breathing instructions. The resulting chemically-driven increase in ventilation should occur through drive to the motoneurons of the respiratory muscles from centres in the medulla (39). It should not require attention or output from the motor cortex to drive respiration (9, 28, 31), although sensations related to breathing will increase. Third, subjects used feedback to match the hypercapnic hyperpnea while end-tidal CO₂ was held at a normal level. This task required attention and high levels of output from the motor cortex to respiratory muscles. We expected that this would result in a deficit in drive from the motor cortex to the arm muscles. Finally, when no deficits in arm performance were seen with the matched voluntary hyperpnea, subjects were set ventilatory targets with high rates and a volume equivalent to their vital capacity. Thus, this breathing task required close to maximal efforts in inspiration and expiration. A slight decline in minute ventilation over the 2 min MVC was due to a steady decrease in inspiratory volume, and may indicate some fatigue of the respiratory muscles (7, 17,18). Indeed, subjects complained of sore abdominal (expiratory) muscles at the conclusion of the experiment. However, the combination

of near maximal respiratory efforts with a fatiguing sustained maximal effort of the elbow flexors still failed to elicit a deficit in arm performance.

Confirming the lack of change in force and voluntary activation in the elbow flexion task, there were no robust changes in EMG measures between conditions. Motor evoked potentials (MEPs) and silent periods were not altered by hypercapnia. For biceps and brachioradialis, MEPs grew and silent periods lengthened during the 2-min MVC as expected (42). MEPs behaved similarly in all 5 ventilation conditions. The silent period in biceps lengthened more on Day 1 than on Day 2 but this was an isolated finding with no similar finding in brachioradialis and no other differences between conditions. Thus, there was no evidence that changing the neural drive to the respiratory muscles altered the behaviour of the motor cortex driving the elbow flexors.

The surprising lack of interaction between the breathing and elbow flexion tasks may indicate that drive to one muscle group is independent of drive to another muscle group, and that previously observed deficits might be due to biomechanical constraints. However, the result may be specific for the respiratory muscles and may indicate that the neural interactions which occur with work of two limbs do not hold for combined work of the respiratory and limb muscles. Decrements in task performance are not always observed with simultaneous contractions of non-homologous muscles, nor with well-practised contractions, such as bilateral leg extensions in trained weightlifters (23, 24). Although the circumstances in which humans would produce minute ventilations as high as during the final experimental protocol are rare, breathing is nonetheless a well-practised task. Lastly, it is difficult to know whether the same neural effects

should hold for respiratory muscles, as they can be driven by both the cortex and/or the brainstem.

One methodological consideration is that voluntary activation of the elbow flexor muscles was measured using cortical stimulation rather than peripheral nerve stimulation (43, 45). Using cortical stimulation to measure voluntary activation reveals whether, at the moment of stimulation, there is motor cortical output which is untapped by voluntary effort but available for recruitment by the stimulus, and which can produce more force from the muscle. If all available cortical output were engaged voluntarily but this were not enough to drive the muscle maximally, then voluntary activation could be high as measured by cortical stimulation but low if measured by peripheral nerve stimulation (a test of whether the muscle is driven fully). Thus, it would be possible for drive to the muscle to be impaired despite unimpaired voluntary activation as measured with cortical stimulation. However, it is unlikely that this occurred in the current study because, like voluntary activation, subjects' maximal voluntary force was not altered by any of the breathing tasks.

A second consideration is whether the voluntary hyperpnea was truly voluntary. A large increase in ventilation occurred at the start of the 2 min MVC in every condition, mostly due to a tripling of breathing rate. It is under debate whether this exercise hyperpnea is due to a central feedforward command to the cardiorespiratory centres in parallel with the descending command to the motoneurons, or to afferent feedback, for example, from metabolites and changes in muscle tension (e.g. 19, 51). The instantaneous onset of the exercise hyperpnea favours the former interpretation, although both central feedforward and afferent feedback play a role in

ongoing exercise. On the day when subjects' breathing was not controlled, ventilation increased approximately threefold at the start of the 2-min MVC. A similar absolute increase occurred when subjects were hypercapnic but could otherwise breathe freely. Thus, when subjects were required to hyperventilate voluntarily it is likely that this was assisted during the 2 min MVC by an "automatic", exercise-related drive. This was reflected in reports from some subjects that matching the hyperpnea targets became less effortful when the 2 min MVC started, despite the target ventilation itself also increasing. However, subjects' ventilation when attempting target volumes of vital capacity was approximately twice that of uncontrolled breathing so that, at least for this condition, a large proportion of drive to the respiratory muscles remained subject to volition.

Our results differ from studies which show impaired exercise performance following periods of increased respiratory work (e.g. 8, 10, 20, 21, 37). These studies suggest that impaired performances results from decreased blood flow to the exercising muscle, and that blood flow is decreased by a sympathetic reflex response generated by fatigue of the diaphragm (8, 10, 20, 21, 37). Fatigue of the diaphragm was not an aim of our study and the short period of respiratory work probably did not fatigue this resistant muscle (16, 32). In addition, the type of limb exercise performed in our study differed from the experiments reporting a large effect of respiratory work. Most previous studies have looked at dynamic exercise in the legs (cycling or running), in contrast to a static upper limb contraction. The effect of respiratory work on dynamic arm exercise is unclear. Volianitis et al (50) have reported improved rowing performance after a period of inspiratory muscle training while van Houtte et al. (49) reported no change in the endurance time of arm cranking exercise following a period of isocapnic hyperventilation.

Furthermore, if the limit to performance is caused by blood flow interactions, the maximal isometric contraction of our study will have minimized any effect as intramuscular pressure makes such sustained contractions virtually ischaemic (36, 53).

In summary, the study aimed to show that an impairment of exercise performance arose due to an inability to direct neural drive to the exercising muscles and the respiratory muscles simultaneously. In contrast to expectations, cortically-driven breathing had no effect on MVC force or voluntary activation at low, medium and near maximal levels of respiratory work.

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References

1. **Aaron EA, Johnson BD, Seow CK, Dempsey JA.** Oxygen cost of exercise hyperpnea: measurement. *J Appl Physiol* 72: 1810-1817, 1992.
2. **Allen GM, McKenzie DK, Gandevia SC.** Twitch interpolation of the elbow flexor muscles at high forces. *Muscle Nerve* 21: 318-328, 1998.
3. **Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ, Dempsey JA.** Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol* 101: 119-127, 2006.
4. **Babb TG.** Ventilation and respiratory mechanics during exercise in younger subjects breathing CO₂ or HeO₂. *Respir Physiol* 109: 15-28, 1997.
5. **Babb TG.** Ventilatory response to exercise in subjects breathing CO₂ or HeO₂. *J Appl Physiol* 82: 746-754, 1997.
6. **Babb TG, DeLorey DS, Wyrick BL.** Ventilatory response to exercise in aged runners breathing He-O₂ or inspired CO₂. *J Appl Physiol* 94: 685-693, 2003.
7. **Babcock MA, Pegelow DF, Harms CA, Dempsey JA.** Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue. *J Appl Physiol* 93: 201-206, 2002.
8. **Bye PTP, Farkas GA, Roussos C.** Respiratory factors limiting exercise. *Annu Rev Physiol* 45: 439-451, 1983.
9. **Colebatch JG, Adams L, Murphy K, Martin AJ, Lammertsma AA, Tochon-Danguy HJ, Clark JC, Friston KJ, Guz A.** Regional cerebral blood flow during volitional breathing in man. *J Physiol* 443: 91-103, 1991.
10. **Dempsey JA, Romer L, Rodman J, Miller J, Smith C.** Consequences of exercise-induced respiratory muscle work. *Respir Physiol Neurobiol* 151: 242-250, 2006.
11. **Dodd SL, Powers SK, Thompson D, Landry G, J. L.** Exercise performance following intense, short-term ventilatory work. *Int J Sports Med* 10: 48-52, 1989.
12. **Evans KC, Shea SA, Saykin AJ.** Functional MRI localisation of central nervous system regions associated with volitional inspiration in humans. *J Physiol (Lond)* 520 Pt 2: 383-392, 1999.
13. **Eves ND, Petersen SR, Jones RL.** Hyperoxia improves maximal exercise with the self-contained breathing apparatus (SCBA). *Ergonomics* 45: 829-839, 2002.
14. **Gandevia SC.** Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725-1789, 2001.
15. **Gandevia SC, Allen GM, Butler JE, Taylor JL.** Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol (Lond)* 490: 529-536, 1996.
16. **Gandevia SC, McKenzie DK, Neering IR.** Endurance properties of respiratory and limb muscles. *Respir Physiol* 53: 47-61, 1983.
17. **Hamnegård CH, Wragg S, Kyroussis D, Mills GH, Polkey MI, Moran J, Road J, Bake B, Green M, Moxham J.** Diaphragm fatigue following maximal ventilation in man. *Eur Respir J* 9:241-247, 1996.
18. **Kyroussis D, Mills GH, Polkey MI, Hamnegard CH, Wragg S, Road J, Green M, Moxham J.** Effect of maximum ventilation on abdominal muscle relaxation rate. *Thorax* 51:510-515, 1996.

19. **Haouzi P.** Point:Counterpoint: Supraspinal locomotor centers do/do not contribute significantly to the hyperpnea of dynamic exercise. *J Appl Physiol* 100: 1077-1083, 2006.
20. **Harms CA.** Effect of skeletal muscle demand on cardiovascular function. *Med Sci Sports Exerc* 32: 94-99, 2000.
21. **Harms CA, Wetter TJ, McClaran SR, Pegelow DF, Nickele GA, Nelson WB, Hanson P, Dempsey JA.** Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol* 85: 609-618, 1998.
22. **Harms CA, Wetter TJ, St Croix CM, Pegelow DF, Dempsey JA.** Effects of respiratory muscle work on exercise performance. *J Appl Physiol* 89: 131-138, 2000.
23. **Herbert RD, Gandevia SC.** Muscle activation in unilateral and bilateral efforts assessed by motor nerve and cortical stimulation. *J Appl Physiol* 80: 1351-1356, 1996.
24. **Howard JD, Enoka RM.** Maximum bilateral contractions are modified by neurally mediated interlimb effects. *J Appl Physiol* 70: 306-316, 1991.
25. **Iandelli I, Aliverti A, Kayser B, Dellaca R, Cala SJ, Duranti R, Kelly S, Scano G, Sliwinski P, Yan S, Macklem PT, Pedotti A.** Determinants of exercise performance in normal men with externally imposed expiratory flow limitation. *J Appl Physiol* 92: 1943-1952, 2002.
26. **Latash ML, Li S, Danion F, Zatsiorsky VM.** Central mechanisms of finger interaction during one- and two-hand force production at distal and proximal phalanges. *Brain Res* 924: 198-208, 2002.
27. **Lorist MM, Kernell D, Meijman TF, Zijdwind I.** Motor fatigue and cognitive task performance in humans. *J Physiol (Lond)* 545: 313-319, 2002.
28. **Macefield G, Gandevia SC.** The cortical drive to human respiratory muscles in the awake state assessed by premotor cerebral potentials. *J Physiol* 439: 545-558, 1991.
29. **Mador MJ, Acevedo FA.** Effect of respiratory muscle fatigue on subsequent exercise performance. *J Appl Physiol* 70: 2059-2065, 1991.
30. **Martin B, Heintzelman M, Chen HI.** Exercise performance after ventilatory work. *J Appl Physiol* 52: 1581-1585, 1982.
31. **McKay LC, Evans KC, Frackowiak RS, Corfield DR.** Neural correlates of voluntary breathing in humans. *J Appl Physiol* 95: 1170-1178, 2003.
32. **McKenzie DK, Bigland-Ritchie B, Gorman RB, Gandevia SC.** Central and peripheral fatigue of the human diaphragm and limb muscles assessed by twitch interpolation. *J Physiol (Lond)* 454: 643-656, 1992.
33. **O'Donnell DE, D'Arsigny C, Raj S, Abdollah H, Webb KA.** Ventilatory assistance improves exercise endurance in stable congestive heart failure. *Am J Respir Crit Care Med* 160: 1804-1811, 1999.
34. **Ohtsuki T.** Decrease in human voluntary isometric arm strength induced by simultaneous bilateral exertion. *Behav Brain Res* 7: 165-178, 1983.
35. **Romer LM, Lovering AT, Haverkamp HC, Pegelow DF, Dempsey JA.** Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. *J Physiol (Lond)* 571: 425-439, 2006.
Physiol Neurobiol 156: 353-361, 2007.
36. **Sadamoto T, Bonde-Petersen F, Suzuki Y.** Skeletal muscle tension, flow, pressure, and EMG during sustained isometric contractions in humans. *Eur J Appl Physiol Occup Physiol* 51: 395-408, 1983.

37. **Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA.** Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol (Lond)* 537: 277-289, 2001.
38. **Sliwinski P, Yan S, Gauthier AP, Macklem PT.** Influence of global inspiratory muscle fatigue on breathing during exercise. *J Appl Physiol* 80: 1270-1278, 1996.
39. **Smith CA, Ainsworth DM, Henderson KS, Dempsey JA.** Differential responses of expiratory muscles to chemical stimuli in awake dogs. *J Appl Physiol* 66: 384-391, 1989.
40. **Spengler CM, Knopfli-Lenzin C, Birchler K, Trapletti A, Boutellier U.** Breathing pattern and exercise endurance time after exhausting cycling or breathing. *Eur J Appl Physiol* V81: 368-374, 2000.
41. **Tabachnick BG, Fidell LS.** *Using multivariate statistics.* New York: HarperCollins, 1996.
42. **Taylor JL, Butler JE, Allen GM, Gandevia SC.** Changes in motor cortical excitability during human muscle fatigue. *J Physiol (Lond)* 490: 519-528, 1996.
43. **Taylor JL, Todd G, Gandevia SC.** Evidence for a supraspinal contribution to human muscle fatigue. *Clin Exp Pharmacol Physiol* 33: 400-405, 2006.
45. **Todd G, Butler JE, Taylor JL, Gandevia SC.** Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol (Lond)* 563: 621-631, 2005.
45. **Todd G, Taylor JL, Gandevia SC.** Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol (Lond)* 551: 661-671, 2003.
46. **Todd G, Taylor JL, Gandevia SC.** Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. *J Appl Physiol* 97: 236-242, 2004.
47. **Turner D.** Expiratory resistive loaded breathing in humans increases fluctuations of force production in submaximal isometric quadriceps contractions. *Neurosci Lett* 328: 13-16, 2002.
48. **van Dieen JH, Ogita F, De Haan A.** Reduced neural drive in bilateral exertions: a performance-limiting factor? *Med Sci Sports Exerc* 35: 111-118, 2003.
49. **van Houtte S, Verellen J, Gosselink R, Vanlandewijck YC.** Influence of isocapnic hyperpnoea on maximal arm cranking performance. *Eur J Appl Physiol* 90: 581-587, 2003.
50. **Volianitis S, McConnell AK, Koutedakis Y, McNaughton L, Backx K, Jones DA.** Inspiratory muscle training improves rowing performance. *Med Sci Sports Exerc* 33: 803-809, 2001.
51. **Waldrop TG, Iwamoto GA.** Point:Counterpoint: Supraspinal locomotor centers do/do not contribute significantly to the hyperpnea of dynamic exercise. *J Appl Physiol* 100: 1077-1083, 2006.
52. **Walsh ML, Takeda C, Takahashi A, Ikeda Y, Endo M, Miura A, Kan A, Fukuba Y.** Volitional hyperventilation during ramp exercise to exhaustion. *Applied Physiology, Nutrition and Metabolism* 31: 211-217, 2006.
54. **Wigmore DM, Propert K, Kent-Braun JA.** Blood flow does not limit skeletal muscle force production during incremental isometric contractions. *Eur J Appl Physiol* 96: 370-378, 2006.

Table 1. Breathing variables on each day.

	Day 1 (Control)	Day 2 (Matched Control)	Day 3 (CO₂-driven Hyperpnea)	Day 4 (Voluntary Hyperpnea)	Day 5 (Maximal Voluntary Hyperpnea)
During rest between brief control contractions of elbow flexor muscles on each day.					
Ventilation (L/min)	12.6±3.0	12.6±2.3	27.3±7.0	25.4±6.9	55.6±14.5
(%MVV)	(11±3)	(11±2)	(24±8)	(23±7)	(49±13)
V _t (l)	0.92±0.30	0.93±0.29	1.68±0.34	1.56±0.33	3.24±0.65
(%VC)	(23±6)	(24±5)	(44±10)	(40±9)	(82±6)
T _i (s)	1.52±0.37	1.39±0.32	1.55±0.30	1.45±0.26	1.46±0.31
T _e (s)	2.95±0.66	3.05±0.77	2.29±0.64	2.38±0.73	2.20±0.79
Frequency (breaths/min)	14.3±3.0	14.2±3.2	16.5±4.1	16.5±4.1	17.5±4.4
During the 2 minute sustained maximal contraction of elbow flexor muscles on each day.					
Ventilation (L/min)	45.5±12.2	46.3±13.2	64.8±13.3	57.7±11.2	87.6±13.0
(%MVV)	(41±13)	(41±13)	(58±16)	(51±12)	(77±11)
V _t (l)	1.35±0.41	1.32±0.30	1.81±0.49	1.63±0.44	2.76±0.57
(%VC)	(35±11)	(34±8)	(47±13)	(42±11)	(70±7)
T _i (s)	0.77±0.31	0.72±0.17	0.78±0.23	0.77±0.19	0.89±0.19
T _e (s)	1.17±0.63	1.16±0.74	0.95±0.39	0.98±0.45	1.05±0.39
Frequency (breaths/min)	36.7±11.8	36.8±11.8	37.7±8.8	37.2±9.5	32.9±7.1

Mean ± SD ventilation expressed also as a percentage of maximum voluntary ventilation (%MVV), tidal volume (V_t) expressed also as a percentage of vital capacity (VC), inspiratory time (T_i), expiratory time (T_e) and frequency of breathing, and end-tidal CO₂ measured across the 2 minute maximal contraction of elbow flexors on each experimental day (Days 1-5) where ventilation was varied. * Ventilation was significantly higher on Days 3-5 compared to Days 1 and 2, on Day 3 compared to Day 4, and on Day 5 compared to Day 3.

Figure Legends

Figure 1. Diagram of experimental protocol. A. schedule of contractions for each day. Subjects performed sets of brief contractions to 100%, 75% and 50% MVC, breathing room air (Controls) and breathing through the mouthpiece (Breathing controls). They performed 3 sets of contractions under each condition. Cortical (solid arrows) and brachial plexus stimuli (dashed arrows) were delivered 1s apart during the brief MVCs and cortical stimuli were delivered during the 75% and 50% MVCs. Subjects then performed a 2 min MVC, with cortical and brachial plexus stimuli delivered 5 s apart every 20 s. Five further sets of brief contractions were performed during recovery. **B. Diagram of the breathing protocols for each day.** On the Control day, subjects received no breathing instructions. For the Matched Control day, subjects were provided with targets for inspiratory time (T_i), expiratory time (T_e) and tidal volume, while the experimenter maintained CO_2 at Day 1 levels. On the CO_2 -driven Hyperpnea day, the experimenter maintained a high CO_2 level, but subjects received no breathing instructions. The inspiratory and expiratory times and the tidal volume from this day were used as feedback for the Voluntary Hyperpnea day, when the experimenter maintained CO_2 at normal levels. For the Maximal Voluntary Hyperpnea day, subjects were provided with targets of the inspiratory and expiratory times from Day 3, and their vital capacity as the target volume, while experimenters kept the CO_2 at normal levels.

Figure 2. Ventilation (A) and end-tidal CO_2 (B) over the control period, 2 min MVC and recovery period during 5 different breathing protocols for the group of subjects (n=8).

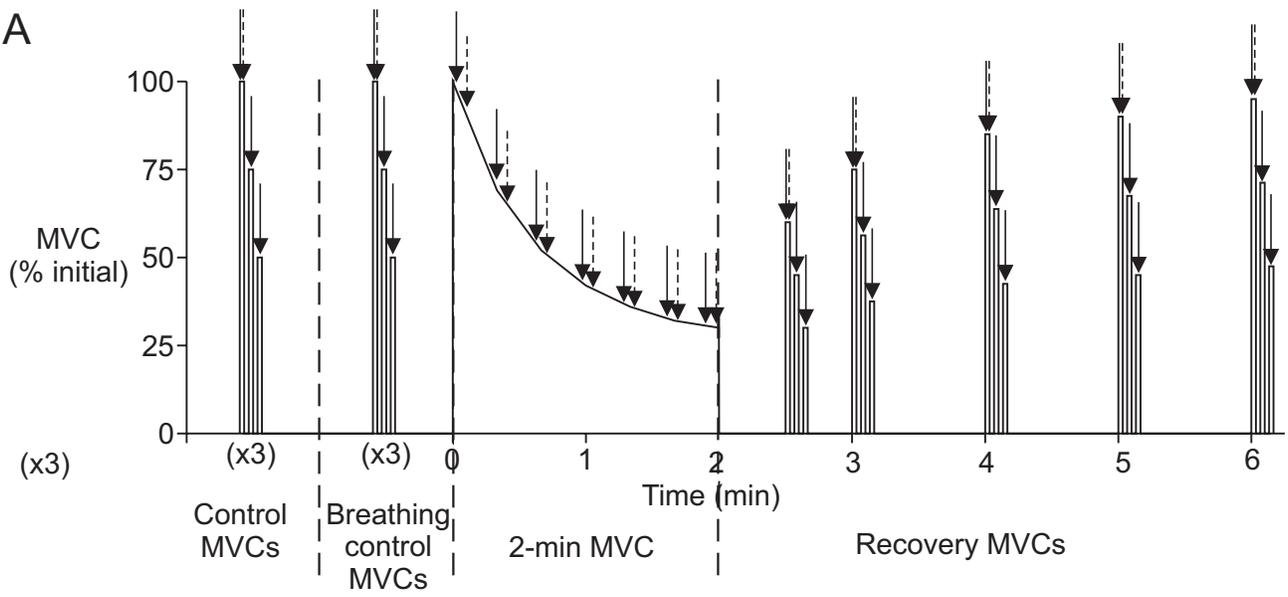
Minute ventilation (L/min) is normalized to the Day 1 control period. Means \pm SEM are shown. The 5 breathing protocols were performed on different experimental days: Day 1 (filled circles), normal breathing or Control; Day 2 (open triangles), Matched Control; Day 3 (filled square),

CO₂-driven Hyperpnea; Day 4 (open diamond), Matched Voluntary Hyperpnea; and Day 5 (filled triangle), Maximal Voluntary Hyperpnea.

Figure 3. Voluntary force and superimposed twitch force during maximal voluntary contractions of the elbow flexors during 5 different breathing protocols for a group of subjects (n=8). **A:** Voluntary force in brief control MVCs, during a 2 min MVC and in brief MVCs in the recovery period. Prior to the 2-min MVC, brief control MVCs were performed during normal breathing (MVC controls) and with breathing according to the day's protocol (Breathing controls). Forces were normalized to the MVCs performed during normal breathing for each day. Mean±SEM are shown. **B:** Superimposed twitch force elicited by cortical stimulation in brief MVCs in the control periods, during a 2-min MVC and in brief MVCs in the recovery period. The amplitude of the superimposed twitch is expressed relative to the ongoing maximal voluntary force measured just prior to each stimulus. Mean±SEM are shown. The 5 breathing protocols were performed on different experimental days: Day 1 (filled circles), normal breathing or Control; Day 2 (open triangles), Matched Control; Day 3 (filled square), CO₂-driven Hyperpnea; Day 4 (open diamond), Matched Voluntary Hyperpnea; and Day 5 (filled triangle), Maximal Voluntary Hyperpnea.

Figure 4. Force traces showing superimposed twitches elicited by cortical stimulation from a single subject on the Control day (normal breathing) and during Maximal Voluntary Hyperpnea. Twitches were evoked in brief MVCs during normal breathing (MVC controls) and while the subject breathed according to the day's protocol (Breathing controls), during the 2 min MVC, and in brief MVCs in the recovery period. Arrows and vertical broken lines indicate the time of stimulation of the motor cortex.

A



B

