Neuromuscular Fatigue during Sustained Contractions Performed in Short-Term Hypoxia

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ABSTRACT

SZUBSKI, C., M. BURTSCHER, and W. N. LÖSCHER. Neuromuscular Fatigue during Sustained Contractions Performed in Short-Term Hypoxia. Med. Sci. Sports Exerc., Vol. 39, No. 6, pp. 948–954, 2007. Purpose: Hypoxia is known to change neuronal activity in vitro and to impair performance in vivo. The present study was designed to study neuromuscular fatigue in acute hypoxia, and we hypothesized that hypoxia results in additional fatigue during sustained contractions, presumably because of increased central fatigue. Methods: Twelve healthy subjects participated in a normoxic (NX) and hypoxic (HX) experiment performed on separate days. Hypoxia was induced by breathing an HX air mixture containing 12% oxygen. Before, during, and after a 90-s sustained voluntary maximal contraction (MVC) of the first dorsal interosseus muscle, we measured force, voluntary activation (VA), and parameters of motor cortical excitability (motor-evoked potentials (MEP) and silent periods (SP)). Measures of peripheral nerve and muscle function, compound motor action potential (M-wave), and muscle twitch forces were also taken. Results: During the MVC, force declined similarly during both HX and NX. VA decreased throughout the contraction in HX, but, surprisingly, this decrease in VA in HX did not exceed that observed in NX. Also, motor cortical excitability changed to a similar degree in HX and NX; that is, MEP amplitude and SP duration increased. M-wave amplitude decreased significantly during the sustained MVC in NX and HX. The only difference observed between NX and HX was the quicker recovery of the muscle twitch in HX, which was even potentiated after 5 min of recovery. Conclusion: The present results show that peripheral and central neuromuscular adaptations during a sustained fatiguing contraction are similar in NX and HX. The quicker recovery and potentiation of twitch forces in HX suggest alterations in myosin phosphorylation, which may enhance contractile force. Key Words: TRANSCRANIAL MAGNETIC STIMULATION, CENTRAL FATIGUE, MOTOR CORTEX EXCITABILITY, VOLUNTARY ACTIVATION

Central neurons require continuous and sufficient delivery of oxygen to enable vital processes within cells. Thus, central neurons quickly sense reduced availability of oxygen and change their activity in response to hypoxia (HX) (5,32), and the hypothesis of a “central governor” regulating muscle activity during exercise in HX was formulated (33).

Numerous in vivo studies on the effects of HX on the central nervous system (CNS) have focused their attention primarily on mental performance such as attention or mood, demonstrating deficits in cognitive performance attributable to exposure to high altitude (29,30). Likewise, similar deficits in verbal and cognitive function have been observed in patients with severe chronic obstructive pulmonary disease (COPD) compared with the normal population (21). These indications of impaired CNS function in HX are supported by studies on psychomotor skills that have demonstrated increased visual and auditory reaction times during HX (9,10).

It has been shown that HX does not impair force production during brief maximum voluntary contractions (14,35). However, during prolonged fatiguing contractions, conflicting results have been reported. Whereas some have found no effects of HX on force decline during sustained contractions (8,18), others have reported greater fatigue in HX (14,15). Also, little is known about motor cortex physiology during fatigue in HX. In normoxia (NX), motor cortex excitability increases during fatiguing contractions while, at the same time, central fatigue develops, which has been shown to be attributable to a suboptimal drive of the motor cortex (17,36). Changes in neurotransmitters function are suggested to be involved in mechanisms mediating central fatigue (16,22). Because HX is also thought to impair neurotransmitter function (23,38), and because HX has been shown to modify motor cortex excitability (35), we hypothesized that fatigue in HX results in additional adaptations of motor cortex excitability, resulting in increasingly central fatigue. This study was, therefore,
designed to examine motor cortical excitability during a fatiguing contraction under an HX condition, using transcranial magnetic stimulation (TMS).

METHODS

Subjects

Twelve right-handed healthy male volunteers, aged 21–45 yr, participated in two experiments performed on separate days. Cardiovascular health was ascertained before the experiments by ECG and medical examination. Subjects were asked to avoid finger- and hand-muscle exercises for at least 24 h before the experiments. All subjects gave written informed consent to the experiments and the study was approved by the ethics committee, Innsbruck Medical University. All experiments conformed to the Declaration of Helsinki.

Experimental Protocol

Subjects were introduced to the experimental apparatus, and they practiced submaximal and maximal index-finger abductions. Then, three electrical nerve stimulations were delivered at intervals of 5 s while the muscle was relaxed. Thereafter, the subjects performed six brief control maximum voluntary contractions (MVC) lasting 3 s each and separated by 1 min of rest. During each MVC, a stimulus to the nerve or to the motor cortex was delivered at peak voluntary force, and subjects were instructed to continue the MVC for another second after stimulation (Fig. 1A). Then, a 90-s fatiguing MVC was performed, during which four pairs of stimulations were given every 20 s. Each pair involved nerve stimulation and TMS at 2-s intervals (Fig. 1B). This stimulation protocol was started 2 s after the onset of the fatigue task. Throughout the experiment, force was displayed on a computer monitor for visual feedback, and subjects were verbally encouraged to produce MVC throughout the sustained contraction. After the fatiguing MVC, further stimulations were given, and MVC were performed to monitor the recovery period. A stimulation series consisted of nerve stimulations at rest and during a brief MVC, followed by TMS during a second MVC trial, with 4 s of rest between the MVC. These measures were taken 5 s, 30 s, 2 min, and 5 min after the sustained contraction (Fig. 1B). Each subject took part in an NX and an HX session, performed in a randomized order on different occasions, separated by at least 1 wk.

Experimental Setup/Force and EMG Recordings

Each subject was comfortably seated in a semireclined position in an armchair, with the right elbow flexed at an elbow angle of 120° and the pronated forearm placed in a molded armrest. The thumb and index finger were attached to separate metal bars, with an internal angle of 90°. These bars were molded to prevent any flexion movements. To measure index-finger abduction force, the bar restraining the second digit contained a one-dimensional load cell (Megatron KM 2000, Eltroma, Vienna, Austria), which was adjusted individually to the interphalangeal joint in each subject. Fingers 3–5 were separated from the index finger by an additional brace.

Myoelectric activity (EMG) was recorded continuously from the right first dorsal interosseus muscle (FDI) using Ag-AgCl disposable surface electrodes. After shaving and swabbing the skin with isopropyl 70% alcohol, the electrodes were placed in a belly-to-tendon configuration. All signals were amplified and filtered (force, 0–100 Hz; EMG, 53 Hz–5 kHz) using Neurolog amplifiers and filters (Digitimer Ltd., Welwyn Garden City, UK) and A/D converted at 10 kHz (CED 2501, Micro 1401, Cambridge Electronics Design, England).

Stimulation Procedure

Electrical stimulations of the ulnar nerve were performed to assess contractile properties and neuromuscular transmission, and TMS was delivered to the FDI motor cortex to determine motor-evoked potential (MEP), silent period (SP), and voluntary activation (VA) during a sustained maximal index-finger abduction and during recovery.
Peripheral nerve stimulation. The ulnar nerve was stimulated using surface electrodes placed 4 and 6 cm proximal to the right wrist. Stimulations (200-μs duration) were delivered by a constant-current stimulator (DS7AH, Digitimer Ltd., Welwyn Garden City, UK). Stimulating the ulnar nerve at the wrist also activates muscles acting as antagonists to the FDI, which could possibly reduce twitch amplitude and shape. However, these effects would not differ between NX and HX. The stimulation intensity was gradually increased during muscle relaxation until the compound muscle action potential (M-wave) did not increase further. To ensure supramaximal stimulation, the stimulation intensity was then increased by 50%. Double supramaximal stimuli with an interstimulus interval of 20 ms were applied to measure twitch force amplitude. A double pulse was used to produce a larger, more easily measured force response than that evoked by a single stimulus. These stimulations were also used to record M-waves; the first M-wave was used for statistical analysis.

TMS. TMS were delivered via a figure-8 coil with a mean loop diameter of 9 cm by a Magstim 200 (Magstim Dyfed, UK). The coil was held tangentially to the skull, with the handle pointing 45° posterolaterally, and was placed over the left FDI motor cortex area. The optimal coil position was marked directly on the scalp, and the resting motor threshold (rMT), defined as the stimulation intensity necessary to evoke MEP of at least 50 μV in 5 of 10 trials, was determined in the relaxed right FDI. A stimulation intensity of 140% rMT was then used throughout the experiment.

HX

HX was induced by breathing an HX air mixture via a face mask (Hypoxic OHG, Germany). To ensure appropriate adaptation, the fraction of inspired oxygen in nitrogen (FIO₂) was gradually reduced until an FIO₂ of 12% was reached. The experimental stimulation protocol was started after 20–30 min of HX air inhalation, because a stable reduction of blood oxygen saturation (SaO₂) is achieved after this time period (2). Total time spent under the HX condition was about 45 min. A constant 12% O₂ mixture was retained throughout the experiment by connecting the inspiratory tube to a 20-L bag. Arterial oxygen saturation and pulse rate were continuously monitored by an oxymeter placed at the left index finger (Onyx, Nonin Medical Inc.). In NX, subjects also wore a mask, which was detached from the air bag, and they waited 20–30 min before the experiment started.

Data Acquisition and Processing

During all MVC, the maximal force was measured and averaged for 100 ms before TMS stimulation. Peak resting twitch-force amplitude (Fig. 2B), peak-to-peak M-wave amplitude (Fig. 2A), and peak-to-peak MEP amplitudes were measured (Fig. 2C). To account for fatigue-induced changes of neuromuscular transmission, each MEP response was normalized to the amplitude of the M-wave throughout the sustained MVC and recovery period. The duration of the silent period (SP) was determined manually from the time of stimulation to return of continuous voluntary EMG (Fig. 2C). Voluntary activation (VA) was assessed by the force increment evoked by TMS (Fig. 2D), expressed as the percentage of the mean voluntary force for 100 ms preceding the stimulation (VA (%) = (1 – (superimposed twitch/background force)) × 100).

Statistical Analysis

Normality of data distribution was assured using the Shapiro-Wilk W test. During the fatiguing contraction and recovery, all responses were normalized for each subject to their respective control values before the sustained MVC and data were (force, M-wave, twitch, MEP, normalized MEP, SP, and VA) analyzed using a two-way repeated-measures ANOVA (factor: time and condition), and post
**RESULTS**

$\text{SaO}_2$ decreased from 97.00 ± 0.60% in NX to 75.25 ± 2.22% in HX ($P \leq 0.01$), and heart rate increased from 70.08 ± 12.10 bpm (NX) to 81.01 ± 13.38 bpm (HX) ($P < 0.01$). Before the fatiguing contraction, amplitudes of twitch, M-wave, and MEP, and duration of SP and VA did not differ significantly between NX and HX (Table 1).

### Force, Muscle Twitch, and M-Wave

The 90-s fatiguing MVC resulted in a significant decline in force output, showing a similar pattern during NX and HX fatigue and recovery. At the end of the sustained contraction, force had dropped to 38.75 ± 9.90% of prefatigue MVC in NX ($F = 98.63, P < 0.001$) and to 46.30 ± 11.45% during HX ($F = 47.40, P < 0.001$). During recovery, force was consistently below the prefatigue baseline values and was significantly reduced at 15 s, 30s, 2 min, and 5 min after fatigue ($P < 0.01$) in NX and HX. After 5 min of recovery, the forces were 83.05 ± 9.98 and 78.99 ± 13.68% of baseline in NX and HX, respectively (Fig. 3A). During fatigue and recovery, force did not differ between NX and HX.

After MVC, the amplitudes of the resting twitch force were significantly reduced in NX (61.70 ± 18.36%) and HX (74.66 ± 35.81%). Twitch amplitudes recovered more quickly in HX compared with NX, and twitch amplitude was significantly greater in HX after 30 s of recovery ($P < 0.05$). After 5 min of recovery, the twitch reached the prefatigue amplitude (103.70 ± 20.35%) in NX, whereas it was potentiated in HX (133.80 ± 51.71%, $P < 0.05$) (Fig. 4B).

Immediately after the sustained MVC, resting M-wave was significantly reduced in NX (88.76 ± 7.60%) and HX (91.06 ± 9.14%), but amplitudes had fully recovered to 101.20 ± 9.56% (NX) and 101.70 ± 8.76% (HX) within 5 min. HX had no effect on M-wave amplitude throughout the recovery period (Fig. 4A).

### MEP Amplitude, Silent Period, and Voluntary Activation

MEP amplitude, normalized to the M-wave, increased significantly during the fatiguing MVC in NX (241.2 ± 142.80%, $P < 0.01$) and HX (196.10 ± 92.53%, $P < 0.01$)
and returned rapidly to its prefatigue value during recovery (Fig. 3C). Again, the changes in MEP amplitude did not differ significantly between NX and HX. During fatigue, SP significantly lengthened NX and HX ($P < 0.01$). At the end of the sustained MVC, the SP duration was $121.40 \pm 17.17\%$ in NX and $129.30 \pm 19.59\%$ in HX. SP durations recovered rapidly during NX and HX to prefatigue levels within 5 min, returning to 104 and 108% of the prefatigue values, respectively (Fig. 3D). Neither during the sustained MVC nor recovery did SP differ significantly between HX and NX.

During the fatigue protocol, VA declined significantly in NX to $80.67 \pm 10.72\%$ ($P < 0.001$) and in HX to $77.75 \pm 15.58\%$ ($P < 0.001$) toward the end of the sustained contraction, but VA did not differ between NX and HX (Fig. 3B). Also, VA recovered within 15 s in NX and HX.

**DISCUSSION**

In contrast to our initial hypothesis, the results from the present study demonstrate that acute HX has no impact on central and peripheral neuromuscular adaptations that are known to occur during fatiguing contractions performed in a normal environment (16).

In NX, force dropped during the sustained MVC to 39% of its initial value, and a comparable gradual decline in force was observed during the sustained MVC in HX. Previous studies on fatigue during sustained contractions in HX conditions have yielded conflicting results. Whereas some studies have failed to show differences in force decrements between NX and HX (8,18), others have demonstrated increased fatigability in response to HX (14,15). Because these studies investigated different muscles and applied various experimental protocols, this indicates that the concept of task specificity of neuromuscular fatigue (7) is not only valid for fatigue in normal environments; it also holds true for fatigue in HX conditions.

Previous studies have demonstrated that central failure occurs during sustained contractions attributable to reduced voluntary drive to the contracting muscle; this is partly attributable to fatigue at supraspinal levels, “upstream” of the motor cortex (17,31,36). Because *in vitro* studies have shown that HX impairs central neuronal function (3,25), we hypothesized that HX causes additional central fatigue during sustained muscle contraction. In both experimental conditions, voluntary activation decreased, and central fatigue thus occurred, consistent with previous NX studies that have demonstrated that part of the force decline during fatiguing contractions is attributable to suboptimal output from the motor cortex (17,36). However, the decline in voluntary activation in HX was the same as that in NX. These results demonstrate that, in contrast to other acute environmental challenges, such as hyperthermia (37), acute HX does not result in additional central fatigue. Because TMS also activates antagonists to the FDI, the absolute amount of voluntary activation might have been over-estimated using this methodology (16), but it is unlikely that antagonist activation is different in HX. Thus, the relative comparison of voluntary activation between NX and HX should be valid.

Previously, we have shown that acute HX can alter motor cortex excitability (35). On the basis of these observations, we assumed the motor cortex adaptations during fatiguing contractions would differ between NX and HX. During the fatiguing contraction in NX and HX, the amplitude of the cortical MEP increased and the duration of the cortical silent period lengthened, consistent with previous NX findings (17,36). Whereas the increase in MEP amplitude represents increased cortical excitability (4,16), the prolonged duration of the cortically evoked SP reflects increased inhibition of the motor cortex (28). Thus, motor cortex adaptations during neuromuscular fatigue, including excitatory and inhibitory events, were not affected by acute HX.

In addition to central adaptations, we also studied the effects of acute HX on peripheral fatigue. M-wave amplitude declined during the sustained MVC in NX, which has been attributed to neuromuscular junction failure or to decreases in sarcolemmal excitability (11,34), but it recovered quickly after the sustained MVC. Similar results have been reported in a number of studies in NX for the FDI (12,13,39). In the present study, the decline in M-wave...
amplitude and its recovery in HX were similar to that in NX, demonstrating (similar to previous studies (6,18)) that HX does not result in additional impairments of neuromuscular transmission or sarcocellular excitability during fatiguing MVC.

The twitch force measured immediately after the sustained MVC also did not differ between HX and NX, but the twitch recovered more quickly in HX and was even potentiated compared with the prefatigue twitch after 5 min of recovery. Augmented twitch forces resulting from preceding brief muscle contractions have already been demonstrated in a number of NX studies; this phenomenon is known as postactivation potentiation (PAP) (24). PAP has been attributed to phosphorylation of myosin regulatory light chains, which makes actin and myosin more sensitive to Ca\(^{2+}\) (20,27). The occurrence of PAP depends on the characteristics of the prior contraction, and MVC lasting 10–30 s show the greatest PAP (1). Although fatigue and PAP have opposing effects on twitch force, both mechanisms can occur. Short-term fatigue of a 60-s MVC resulted in reduced twitch immediately after the contraction, but it recovered in 3–6 min and was even potentiated by 20–34% (19,26). The contractions performed in the present study lasted longer, but a potentiated twitch was already observed after 5 min of recovery in HX, but not in NX. Because M-wave recovery was the same in HX and NX, the greater twitch amplitude observed during recovery in HX suggests either that contractile properties recover more quickly or that PAP is larger in HX than in NX.

In conclusion, the results of the present study show that neuromuscular adaptations to sustained MVC are essentially the same whether these contractions are performed in a normal environment or in acute HX.

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