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Electromyographic signature of isometric squat in the highest refuge in Europe

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Abstract

Reports of electromyography during hypoxic exercise are contrasting, due to protocol and muscle diversity. This work aimed to investigate alterations in muscle activation and myoelectrical fatigue during exercise at high-altitude in those muscles primarily involved in trekking. Eight young adults balanced by gender and age were tested at low (1667 m) and high (4554 m, "Capanna Margherita", Italy) altitude, during an isometric squat lasting 60 seconds. High-density surface electromyography was performed from the quadriceps of right limb. The root mean square (RMS), median frequency with its slope, and muscle fiber conduction velocity (MFCV) were computed. Neither males nor females showed changes in median frequency (Med: 36.13 vs 35.63 Hz) and its slope (Med: -9 vs -12 degree) in response to high-altitude trekking, despite a great inter-individual heterogeneity, nor differences were found for MFCV. RMS was not significantly equivalent, with greater values at low altitude (0.385 ± 0.104 mV) than high altitude (0.346 ± 0.090 mV). Unexpected results can be due either to a postural compensation of the whole body compensating for a relatively greater effort or to the inability to support muscle activation after repeated physical efforts. Interesting results may emerge by measuring simultaneously electromyography, muscle oxygenation and kinematics comparing trekking at normoxia vs hypoxia.

Key Words: hypoxia; muscle fatigue; HD-sEMG; high altitude.

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Altitude hypoxia triggers a plethora of physiological adaptations.[1] Among these, the muscle system is affected by acute and chronic adaptive responses.[2] Local oxygen consumption and muscle activation are observed to change in response to hypoxic stressors.[3] In addition to structural and functional changes, depicted by imaging and dynamometry,[4,5] the response of the muscle system to the stress of hypoxia can be non-invasively analyzed by using electromyography (EMG). Indeed, EMG represents a powerful tool for investigating the muscle activity, neuromuscular system control strategies and fatigue manifestation.[6]

In a study reported in 1994, no changes in both root mean square (RMS) and mean frequency of EMG signal at 2, 15, and 40 days after arrival at 5,050 m were reported; authors conducted the analysis on biceps brachii at 20, 40, 60, 80, and 100% of maximal voluntary contraction (MVC), concluding that neither acute nor chronic hypoxia *per se* affected motor unit (MU) activation pattern.[7] More recently,[8] simulated hypoxia (FiO_2 = 15% and FiO_2 = 13%, related to moderate and severe hypoxia, respectively) had no effect on EMG of biceps brachii as measured by root mean square and mean frequency. Another study showed that at a given intensity muscle activation of *quadriceps* is greater when exercising under severe hypoxia, i.e., FiO_2 = 10.8%.[9] However, both peripheral and central fatigability can impair neuromuscular function in response to hypoxia.[10] The role of hypoxia as a possible affecting-factor for muscle fiber conduction velocity seems unclear, with a plethora of effects, if any, reported in literature.[11–13] Based on these results, severe hypoxia affects the MU activation pattern, but the most common trekking expeditions did

not reach those hypoxic levels. Less severe hypoxia does not likely result in EMG alterations, but the results are limited to upper arm muscles, that in a field perspective are less affected by the physical effort of trekking. Therefore, it would be interesting to test those muscles primarily involved in the trekking exercise at the altitudes that many trekkers may face.

Within this background, the current work aimed to depict, if any, alterations in muscle activation and myoelectrical fatigue during a physical effort in response to the combined effect of trekking and high-altitude exposure. To increase the feasibility of testing at altitude hypoxia with an ecological study design, it was chosen as a common muscle task with no special equipment required, exploited by muscles involved in trekking, i.e., isometric body-mass squat with a fixed duration.

Materials and Methods

Study overview, participants, and procedures

All participants signed an informed consent; the study was an ancillary project of wider studies approved by the Ethics Committee of G. D'Annunzio University - Chieti and Pescara (n. 18, 29 July 2021), i.e., of the project "Monte Rosa Exploration & Physiology",[14–16] conducted from August 29 to September 2, 2021, in the Western Alps, Italy, with 15 healthy expeditioners trekking up to Capanna Regina Margherita, the highest Europe's mountain lodge. Briefly, the ascent lasted 4 days, with the following elevation plan: in the 1st day, from 1164 (Alagna Valsesia) to 1667 m (Zar-Senni lodge); in the 2nd day, up to 2370 m (Gabiet lodge); in the 3rd day, up to 3647 (Gnifetti lodge); in the 4th day, up to Capanna Regina Margherita 4554 m. The duration of the daily trek differed across participants, to allow everyone to maintain their own pace. A sub-group of 8 young adults (27.6±3.9 years, 20.9±1.8 kg/m^2) balanced by gender and age was tested at both 1667 m and 4554 m of altitude, i.e., low vs high altitude.[17] None of the participants was accustomed to frequent high altitudes exposure. The project included the bioelectrical impedance analysis (BIA) with HumamIMTouch (DS Medica, Milano, Italy), whose baseline values are presented in Table 1. Briefly, the BIA values herein reported refer to those registered before the expedition at low altitude (1164 m), through a whole-body measurement in supine position with legs and arms slightly abducted, no exercise at least 12 h before the measurement, and fasting state > 3h.

Acute mountain sickness (AMS) occurring at the highest lodge was diagnosed by the Lake Louise AMS score.[18] Vital signs were recorded as peripheral saturation (with APN-100 by Contec Medical Systems Co. Ltd, China),

Table 1. *Description of participants, including habitual sports or physical activity, metrics from anthropometric and bioimpedance analysis (registered at low altitude), and diagnosis of AMS (at high altitude)*

	Sex	Age (years)	Physical activity	*1164 m*			*4554 m*
				BMI (kg/m^2)	PA (degree)	SMI	AMS
MR1	male	30	Climbing	21.57	6.8	9.29	no
MR2	female	23	Various	18.11	5.2	6.59	no
MR5	female	25	Trekking	20.22	/	/	yes
MR9	male	29	Cross-country skiing	23.61	6.7	9.79	no
MR10	female	32	None	20.37	5.4	7.42	yes
MR11	male	24	Soccer	22.03	6.8	9.07	no
MR12	female	33	None	19.17	5.6	6.74	yes
MR15	male	25	None	21.88	6.0	8.88	no
Males	4	27±3		22.27±0.91	6.6±0.4	9.26±0.39	
Females	4	28±5		19.47±1.05	5.4±0.2	6.92±0.44	

BMI: body mass index; PA: bioimpedance phase angle; SMI: skeletal musce index as computed by bioimpedance; AMS: acute mountain sickness

blood pressure, and heart rate (with ABPM50 by ContecMedical Systems Co. Ltd, Cina).
Participants had to perform an isometric squat lasting 60 seconds, maintaining as possible their position until the end of the test, despite the onset and advancement of fatigue. Both the tests (1667 m vs 4554 m) were performed into mountain lodges after at least 2 h from the daily trek. Knee angles during the tests were controlled visually by the operator.

HDsEMG recordings and analysis

During the tests, high-density surface electromyography (HD-sEMG) was acquired from the *quadriceps* of right limb, with one semi-disposal adhesive grid of 64 (over 13 rows × 5 columns) gold-coated electrodes of diameter 1 mm with inter-electrode distance of 8 mm (OT Bioelettronica, Torino, Italy). The skin was prepared by abrasion and cleaning. The electrode matrix was placed longitudinally on the muscle, 10 cm above the superior margin of patella, and fixed to the skin to minimize movement artifacts by using bi-adhesive perforated foam layers (SpesMedica, Battipaglia, Italy), whose cavities were filled with a conductive past (SpesMedica) to optimize the skin-electrode contact. Positioning was consistent between each condition and participant. The grid was additionally covered by two strap bands to ensure the contact. Strap electrodes dampened with water were placed around the ipsilateral (reference electrode) and contralateral (ground electrode) ankle. An expert operator conducted all the acquisitions. The quality of EMG signals was preventively checked by estimation of resting RMS to evaluate the noise level. Raw EMGs were detected in monopolar mode with a sampling frequency of 2000 Hz, filtered (bandpass 10–500 Hz), and converted to digital data using a multichannel amplifier (10-500 Hz, EMG-Quattrocento; OT Bioelettronica). Signals were recorded and furtherly visualized and elaborated by the software OT Biolab+ v1.5.6 (OT Bioelettronica).
The root mean square (RMS) was computed on signals divided into 0.5 s epochs, and the series were visually inspected. The first and last 6 seconds of registrations were removed, and the central 48 seconds were split into three 16-s parts (6–22 s, 22–38 s, and 38–54 s). All the series were graphed and visually inspected. The median frequency was computed on signals divided into 4-s epochs by a Fourier transform analysis. The first and last 4 seconds of registrations were removed and the slope of the graphs was computed by linear regression analysis. RMS is used to depict the amplitude, and frequency changes are used as a surrogate of muscle fatigue, as a decrease in the power spectrum during exercise is due to the slowing of action potential propagation, despite changes in firing behavior could also affect the frequency domain. Our analysis was aimed to compare low vs high altitude acquisitions, not to compare our results with those of other groups since it should be noted that electrode configuration, size and geometry act as filters, thereby modifying the shape and features of the MU action potentials. No method of normalization of sEMG amplitude, such as computation of RMS during the maximal analogous test, was implemented because context factors did not allow us to perform a maximal test in both lodges controlling for all contextual factors. Single-differential signals were computed from the monopolar derivations for each column, were visually inspected, and 3-to-6 channels with clear motor unit action potential propagation without shape change were chosen for the muscle fiber conduction velocity (MFCV) analysis. MFCV was then estimated in time epochs of 5 ms from 8th to 12th seconds of each registration by using an algorithm that allows estimates from multichannel EMG signals. Three values of each registration were

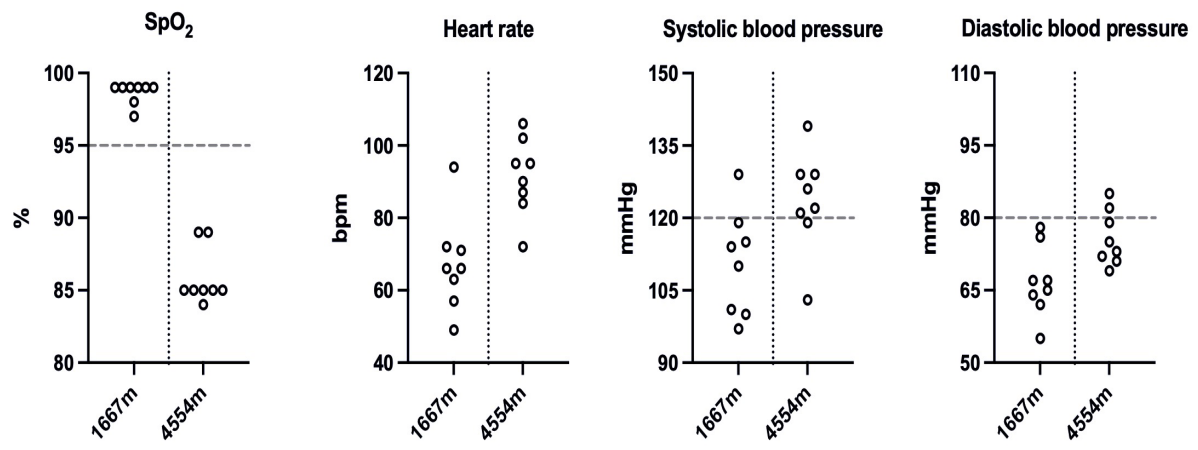

***Fig 1**. Results of vital signs at low and high altitude.*

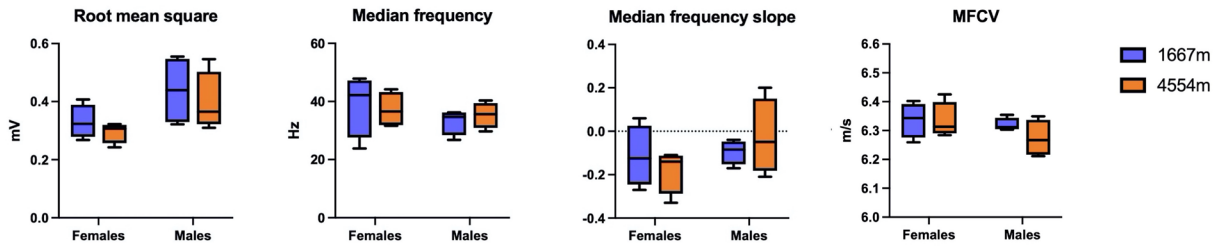

***Fig 2**. Box plots of EMG results at low and high altitude, split by sex. Whiskers stand in the 10–90th percentiles.*

considered after visual inspection of signals and the mean of those three values was considered if CV was < 5%.

Statistics

Statistical analyses were carried out with R-based software Jamovi Version 1.6.23.0 (retrieved by https://www.jamovi.org). Assumptions were checked according to the Shapiro-Wilk test for normality of residuals, evaluation of symmetry and kurtosis, and observation of Q-Q graphs. Comparisons were then conducted with ANOVA for repeated measures (RM-ANOVA), after checking the assumption of homogeneity of variances, setting the altitude as within factor and the sex as between factor. The significance level was set for $p<0.05$ and the effect size was calculated (partial eta squared: η^2_p). The three 16-s segments of RMS were compared by RM-ANOVA with 2 repetition factors (segment and altitude); sphericity was preliminary verified and eventually corrected with the Greenhouse-Geisser method. RMS results were further analysed with the equivalence test (two one-sided t-test: TOST) by TOSTR 0.4.1 package on RStudio 1.1.456; limits were set as –1.156 to 1.156 of effect size, α was set at 0.05 and 1–β at 0.8, with sample size equal to 8. Graphs were created with Prism Version 9 (GraphPad Software, San Diego, USA). SpO_2, HR, and BP were compared with Wilcoxon's rank or Student's t–test in low vs high-altitude. Statistical power and sample size calculation were done on G*Power 3.1.9.3 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The statistical significance was set at $p \leq 0.05$.

***Table 2.** Statistical results of EMG metrics, on the between factor of sex and the interaction of between (sex) × within (altitude) factor*

	p	Effect size
RMS		
Altitude	0.172	n^2_p=0.286
Sex	0.104	n^2_p=0.379
Altitude × Sex	0.906	n^2_p=0.002
Median frequency		
Altitude	0.943	n^2_p=0.001
Sex	0.362	n^2_p=0.140
Altitude × Sex	0.513	n^2_p=0.074
Median frequency slope		
Altitude	0.986	n^2_p=0.000
Sex	0.187	n^2_p=0.270
Altitude × Sex	0.366	n^2_p=0.137
MFCV		
Altitude	0.226	n^2_p=0.276
Sex	0.486	n^2_p=0.251
Altitude × Sex	0.591	n^2_p=0.189

RMS: root mean square; MFCV: muscle fiber conduction velocity

Results

All the participants successfully completed the trek. All of them also completed the exercise task as required for 1 min, at both low and high altitude. Of the eight test subjects, three (all females) developed symptoms of acute mountain sickness; none of them needed therapeutic intervention; the symptoms of AMS had never occurred prior to the high altitude EMG test. We included the participants who developed AMS in the analysis to both represent the condition usually found at that altitude - i.e., about half of the expeditioners develop AMS,[19] - and to account for the female sex, as AMS and female sub-groups almost overlapped (see Table 1)

As shown in Figure 1, the response to high altitude resulted in the expected drop of SpO_2 ($p<0.001$, $r=1$), along with the increase of heart rate ($p<0.001$, Cohen's $d=1.97$) and blood pressure (systolic: $p=0.005$, $d=1.41$; diastolic: $p=0.021$; $r=0.94$).

The high altitude did not significantly affect median frequency, frequency's slope and MFCV (Figure 2 and Table 2). Males and females did not differ significantly in the response to high altitude trekking in the four EMG metrics (Figure 2 and Table 2). The median frequency slope was mainly negative (Figure 2), confirming the expected drop during the 1-minute test as a sign of myoelectrical fatigue.

TOST results showed RMS was not significantly equivalent when comparing low vs high-altitude, with greater values at low altitude (Figure 3). No differences

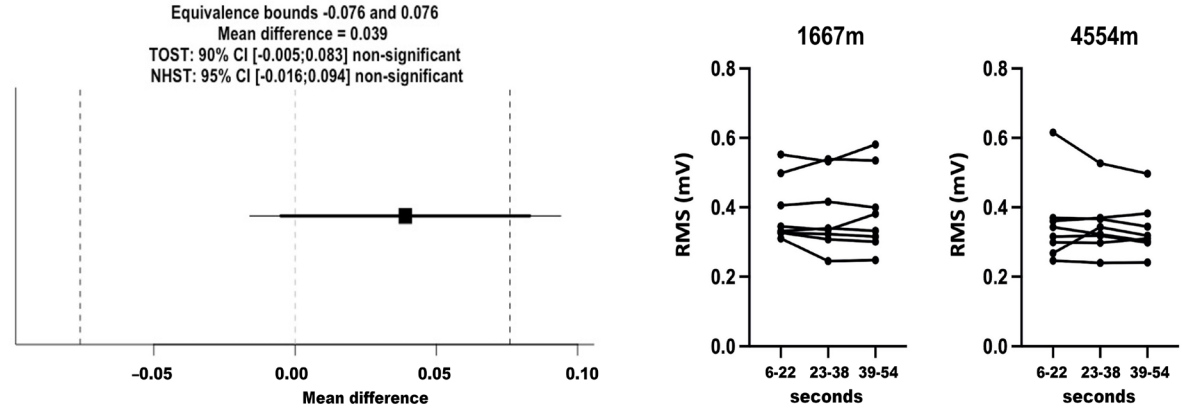

***Fig 3**. TOST results on RMS (left panel) and RMS comparison across three equal segments (right panel).*

appeared across the three segments, nor did the interaction altitude × segment show clear trends (segment: $p=0.592$, $n^2_p=0.061$; altitude × segment: $p=0.511$, $n^2_p=0.074$).

Discussion

Trekking up to high-altitude on a 4-days plans did not affect the manifestation of muscle fatigue on *quadriceps* during an isometric 60-s lasting test, despite this muscle group is primarily engaged during the trekking. RMS was not statistically equivalent when comparing low vs high-altitude test, the latest being featured by lower values. This result does not agree with others reporting no changes in RMS and frequency,[7,8] or greater values of RMS at a given intensity during hypoxic test.[9] In this latest reference, authors discussed their result, suggesting that severe hypoxia, rather than mild, could result in greater RMS values and that the impact of hypoxia is evident if measuring large muscle mass.[9]

During submaximal isometric exercise, both peripheral and supraspinal mechanisms of fatigue may account, with the latest emerging in severe hypoxia, not due to altered corticospinal excitability.[20] However, hypoxia-related mechanisms impair motor cortical output and corticospinal excitability while recovering from fatiguing tasks.[21] It was suggested that an impairment of drive from the motor cortex due to diminished oxygen availability in the brain accounts for the decreasing performance under hypoxia, with an increase in muscle electromyographic activity.[22] If exercising at low intensity under hypoxia, greater supraspinal fatigue does not occur.[23] The performance reduction during exhaustive strength tests can occur with no metabolic and functional muscle states, nor alteration in corticospinal excitability and inhibition; moreover, impaired performance under severe hypoxia is not due to altered afferent feedback, but to mechanisms related to brain oxygenation.[24] Our condition can be considered as mild-to-severe hypoxia and the exercise task as a fatiguing strength test involving large muscle masses, to be performed at a comparable relative intensity. Therefore, based on previous results, an increase in RMS at a high altitude may have been expected. The contrasting slight decrease found can be explained by a preventive mechanism of the whole body, compensating for a relatively greater effort in maintaining the position. Indeed, when studying whole body exercise, biomechanical compensation is always on the stage, accounting for optimizing the muscle chains and supporting fatigued muscles. Further studies investigating intra-muscle synergies will allow to depict whether the motor adjustments during isometric squat in hypoxic conditions selectively affect, if any, either supraspinal or spinal circuitry.[25] Another interpretation can be based on the fact that humans at high altitude could not support the same muscle activation as at low altitude, after a repeated stress (i.e., trekking) on muscles. Following these interpretations, further studies should focus on measuring EMG for comparing 1) exercises on single isolated muscle groups vs whole body exercises, and 2) exercises after a trek in normoxia vs hypoxia. Interesting results may emerge if measuring kinematics during exercise to depict the possible biomechanical compensations and preventive strategies. Other insights of interest may be revealed by integrating sEMG and near-infrared spectroscopy (NIRS) in field studies, as NIRS can assess muscle deoxygenation in response to exercise. Indeed, during an incremental exercise at normobaric hypoxia, despite no changes in EMG metrics occurred, the attenuation of muscle deoxygenation precedes alterations in neuromuscular activity.[26]

Considering that hypoxia is known to affect muscle system,[2,4] other metrics besides RMS, frequency and conduction velocity may serve as either predictive or more favorable variables for muscle impairment. In this regard, it should be of interest to evaluate the force-velocity relationship in response to hypoxia and physical exertion, since shortening velocity adaptations have been suggested as compensatory mechanisms to support power production in inflammatory myopathies.[27] It should be kept in mind that the hypoxia-related cascade

is one of the main non-immune pathological mechanisms in inflammatory myopathies.[28] As an issue limiting the generation of novel insights, despite the use of hd-sEMG matrices, the signals did not allow adequate estimation of single MU contributions. In fact, we planned to use hd-sEMG both to compute RMS and frequency, and to estimate single MUs behaviors.
Unfortunately, the nature of the study, i.e., a field study conducted into a mountain lodge, did not allow to set the test conditions adequately. However, the hd configuration of sEMG improves considerably the reliability of MFCV estimates and of the EMG recordings.[29] Moreover, the free-standing isometric squat limited the control of position, thereby possibly confounding EMG results. Another limit lies on the difference in physical activity type and level across participants, possibly resulting in heterogeneous responses to the hypoxic trekking. All in all, our data confirmed hypoxia has little, if any, effects on the myoelectric activity during an isometric squat, extending similar results on electrical and mechanical activities during sustained maximal isometric contractions in chronic hypoxia.[30]
Prospectively, authors advocate a larger use of sEMG by exercise physiologists aiming to depict the neuromuscular adaptations to extreme environment. In parallel, it should be useful to increase the practical and interpretative skills of scholars and practitioners, such as for EEG in neurology and ECG in cardiology.[31]
Further development of hd-sEMG recording and data analysis will allow a better estimate of muscle behavior in field studies.

List of acronyms

AMS - Acute mountain sickness
EMG - electromyography
HD-sEMG - high-density surface electromyography
MFCV - muscle fiber conduction velocity
MU - motor unit
MVC - maximal voluntary contraction
RMS - root mean square

Contributions of Authors

Conceptualization, DB; Methodology, RR, DB, CS, PP and VV; Formal Analysis, RR and DB; Investigation, DB; Resources, DB, TP and VV; Writing – Original Draft, RR and DB; Writing – Review & Editing, CS, PP, TP, SF, VF, and VV; Visualization, DB; Supervision, TP, SF, VF, and VV; Project Administration, DB and VV; Funding Acquisition, VV. All authors read and approved the final edited typescript.

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Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; data collection, analyses, or interpretation of the data; writing of the manuscript, or in the decision to publish the results.

Ethical Publication Statement

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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