 DOI: https://doi.org/10.5114/biolsport.2021.99322

High-volume intermittent maximal intensity isometric exercise caused great stress, although central motor fatigue did not occur

AUTHORS: Giedre Jurgelaitiene[1], Andrius Satas[1], Agne Cekanauskaite[1], Kristina Motiejunaite[1], Dovile Valanciene[1], Jurate Stanislovaitiene[3], Kristina Bradauskiene[3], Daiva Majauskiene[2], Diana Karanauskiene[2], Albertas Skurvydas[4,5]

[1] Institute of Sport Science and Innovations, Lithuanian Sports University, Sporto str. 6, 44221 Kaunas, Lithuania
[2] Department of Physical and Social Education, Lithuanian Sports University, Sporto str. 6, 44221 Kaunas, Lithuania
[3] Department of Coaching Science, Lithuanian Sports University, Sporto str. 6, 44221 Kaunas, Lithuania
[4] Department of Rehabilitation, Physical and Sports Medicine, Faculty of Medicine, Vilnius University, M. K. Čiurlionio str. 21/27, LT-03101, Vilnius, Lithuania
[5] Education Academy, Vytautas Magnus University, Jonavos str. 66 – 310, 44191 Kaunas, Lithuania

ABSTRACT: To establish whether very high-volume, high-intensity isometric exercise causes stress to the body and how it affects peripheral and central fatigue. Nineteen physically active healthy male subjects (21.2 ± 1.7 years; height – 1.82 ± 0.41 m, body weight – 79.9 ± 4.5 kg; body mass index – 24.3 ± 2.1 kg/m^2) volunteered to participate in this study. They participated in two experiments 3–5 days apart. Each experiment comprised six series of 60-s maximum voluntary contraction (MVC) force (knee extension) achieved as rapidly as possible. This very high-volume, high-intensity exercise (HVHIE) was performed at different quadriceps muscle lengths: short (SL) and long (LL). The MVC and the electrically stimulated contractile properties of the muscle were measured prior to HVHIE, immediately after and 3 min after each series, and at 3, 10, and 30 min after the end of HVHIE. We found that HVHIE caused high levels of stress (cortisol levels approximately doubled, heart rate and the root mean square successive difference of interval (RMSSD) decreased by about 75%); lactate increased to 8–11 mmol/L, voluntary and 100 Hz stimulation-induced force (recorded immediately after HVHIE) decreased by 55% at LL and 40% at SL. However, the central activation ratio during MVC did not change after either exercise. Isometric HVHIE performed using one leg caused high levels of stress (RMSSD decreased, cortisol increased after HVHIE equally at SL and LL; La increased more while exercising at LL) and the voluntary and electrostimulation-induced muscle force significantly decreased, but muscle central activation during MVC did not decrease.

CITATION: Jurgelaitiene G, Satas A, Cekanauskaite A et al. High-volume intermittent maximal intensity isometric exercise caused great stress, although central motor fatigue did not occur. Biol Sport. 2021;38(3)315–323.

Received: 2020-03-13; Reviewed: 2020-06-05; Re-submitted: 2020-07-31; Accepted: 2020-08-27; Published: 2020-10-xx.

Corresponding author:
Dovile Valanciene
Institute of Sports Science and Innovations, Lithuanian Sports University, Sporto str. 6, LT-44221, Kaunas, Lithuania
E-mail: dovile.valanciene@lsu.lt

Key words:
High-volume
High-intensity isometric exercise
Physiological stress
Peripheral and central fatigue
Rate of force development
Muscle length

INTRODUCTION

High-intensity exercises are widely used in sport and health promotion [1, 2]. Exercises performed at maximum intensity cause both peripheral and central fatigue [3, 4, 5, 6, 7], resulting in reduced energy stores in muscles and accumulation of metabolites [8]. This in turn reduces Ca^{2+} release from sarcoplasmic reticulum and myofibrillar Ca^{2+} sensitivity [8, 9, 10] and also reduces the adhesion force and number of myosin transverse bridges [11]. In addition, when exercises are performed isometrically (especially at long muscle length), mechanical damage to the muscle can occur [5, 12].

The decrease in muscle strength during exercise depends on many interrelated factors, including group III/IV afferent signals to the central nervous system (CNS) and cardiovascular system, which affect cortex activation and the sense of effort [13, 14]. The greater the flow of afferent stimuli from the periphery, the greater is the central fatigue. This protects the muscles from further burnout because the individual critical threshold (as a hypothetical construct) of peripheral muscle fatigue is the prevailing factor limiting central motor drive [15, 16, 17].

Classical high-intensity exercise is performed on a stationary bicycle in dynamic mode for 4–12 series of 5–30 s each at maximal intensity [1, 18, 19]. We previously showed that 12 series of 5 s each at maximal intensity caused high levels of metabolic muscle fatigue (peripheral fatigue) [6, 18]. For this study, we chose a very high-volume ,high-intensity exercise (HVHIE) comprising six series every 3 min of 60-s maximal voluntary contraction (MVC) force in an isometric mode. Previously, we used continuous 1- and 2-min MVC [4, 5]. We developed three hypotheses. The first hypothesis proposed that because intense, prolonged exercise activates III/IV afferents, which send signals to the CNS and might "protect" muscles from further fatigue or exhaustion [15, 17], six series of 60-s MVC (i.e., HVHIE) should cause very high central fatigue, while peripheral fatigue should stop increasing after 2–3 series. Extremely high

levels of central fatigue should occur when muscle contraction occurs at long muscle lengths (LL) because, in this case, the muscle develops a larger force and there should be greater and faster peripheral/metabolic fatigue. The second hypothesis proposed that because maximal-intensity isometric exercises at long muscle length result in greater muscle damage than those at shorter muscle length (SL) [5, 9], recovery of muscle strength after HVHIE at LL should be slower than that after HVHIE at SL. The third hypothesis proposed that HVHIE should reduce the rate of force development (RFD) more than MVC, as we speculated that the ability of CNS to maximally activate the muscle in the first milliseconds of contraction might demand more voluntary drive than during MVC (which we believe should lead to a greater decrease in RFD than MVC). The aim of our study was to test these three hypotheses.

MATERIALS AND METHODS

Participants

Healthy male students (n = 19) (21.2 ± 1.7 years; height – 1.82 ± 0.41 m, body weight – 79.9 ± 4.5 kg; body mass index – 24.3 ± 2.1 kg/m^2) volunteered to participate in this study. The participants were physically active but did not take part in any formal physical exercise or sports programme. Each participant read and signed an informed consent form consistent with the principles outlined in the Declaration of Helsinki. The Ethics Committee of the Lithuanian University of Health Sciences approved this study.

Study design

The study design is illustrated in Figure 1. Three to five days prior to the experiment, all subjects were introduced to the study protocol, including muscle electrostimulation (ES) and the need to achieve MVC as rapidly as possible. They were also asked to maintain MVC for 30 s to familiarize themselves with extended-length exercise. All 19 subjects participated in two experiments 3–5 days apart. The protocol of the experiment was as follows: six series of 60-s MVC (knee extension) (6x60 s MVC). Participants performed HVHIE at different quadriceps muscle lengths: SL with one leg on the first day and LL with the other leg the next day. Prior to the HVHIE, immediately after each series, just before the next series, as well as 3, 10, and 30 min after the end of the HVHIE, the contractile properties of the muscle induced by ES and MVC were tested. The rest interval between each series was 3 min. MVC was achieved as rapidly as possible in all cases. Quadriceps femoral muscle electromyographies

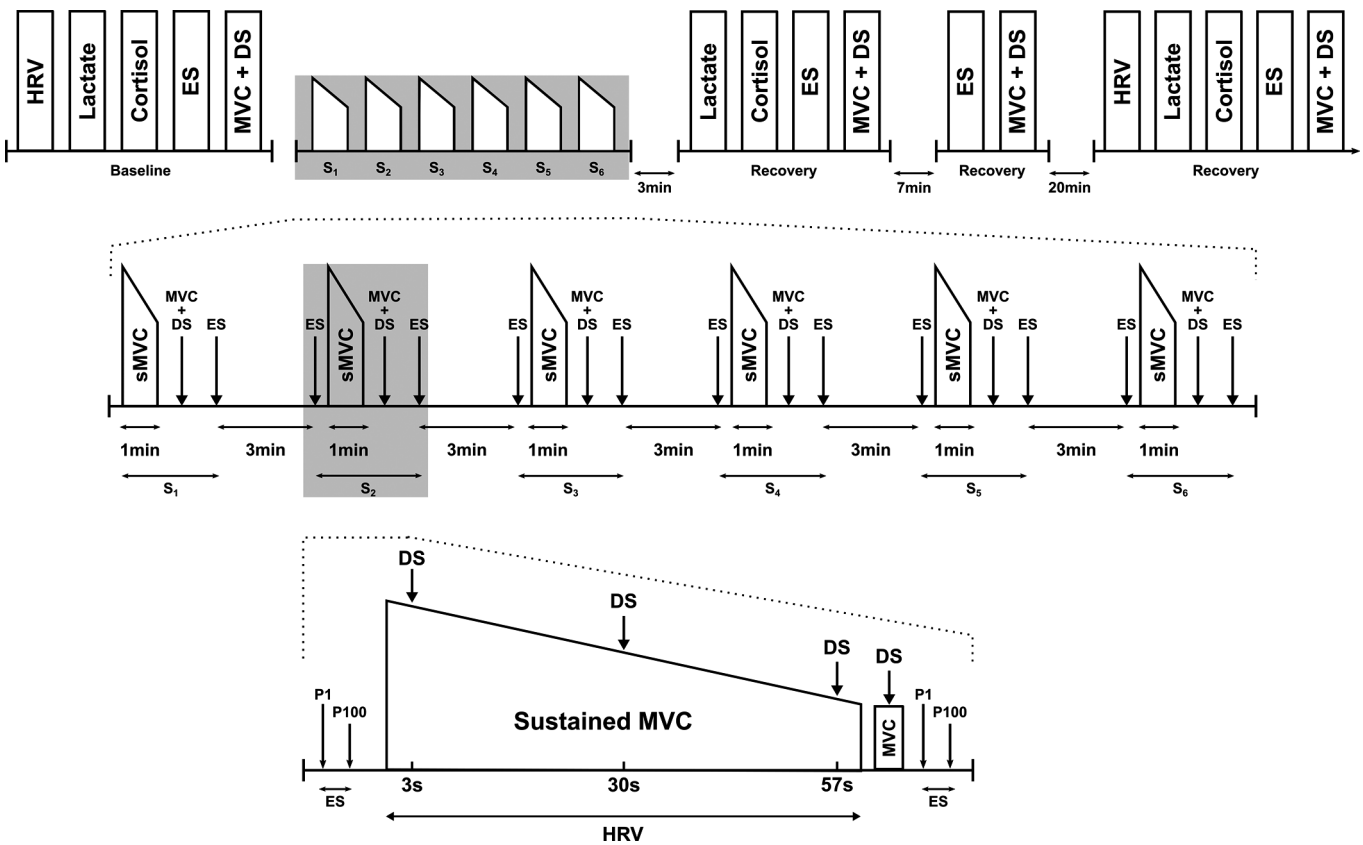

FIG. 1. Study design. HRV – heart rate variability; ES – electrostimulation induced torque at 1 Hz (P1) and 100 Hz (P100); MVC+DS – electrostimulation during maximal voluntary contraction. S_1, S_2, S_3. S_4, S_5 and S_6 – 6 series of maximal voluntary contraction (MVC) of 60 s each. The rest interval between each series was 3 min, i.e. the time period from ES after MVC 60 s to ES before the next MVC.

(EMGs) were recorded throughout HVHIE. In addition, lactate and cortisol concentrations were determined before HVHIE and 3–5 min and 30 min after exercise. Heart rate (HR) was recorded throughout the study.

Measurements

Muscle testing. The subjects were seated in an upright position (hip angle 110°) in a custom chair with adjustable lower-limb attachments and were secured with waist and chest belts. The force produced by the quadriceps of both legs was measured using LPU-100 load cells (Transducer Techniques, Temecula, CA, USA). Force signals were amplified (gain = 40), digitized online using a BIOPAC MP150 data acquisition system (sampling rate 1 kHz), and recorded simultaneously using AcqKnowledge 4.1 software (BIOPAC Systems Inc., Goleta, CA, USA). Subjects were required to keep their arms crossed in front of their chest.

The subject sat upright in the dynamometer chair with the knee joint positioned at 100° (SL) or 145° (LL) (180° = full knee extension). The equipment and procedure for electrical stimulation were essentially the same as previously described [4, 5]. Direct muscle stimulation was applied using two carbonized rubber electrodes covered with a thin layer of electrode gel (ECG-EEG Gel; Medigel, Modi'in, Israel). A standard electrical stimulator (MG 440; Medicor, Budapest, Hungary) was used. The stimulus frequency was 1 Hz (torque – P1) or 100 Hz (torque – P100). ES was delivered in square-wave pulses of 0.5 ms duration. The MVC force was reached as fast as possible, maintained for approximately 3 s before relaxation and measured three times before HVHIE; the highest value was used in the analysis. The time interval between MVC trials was 1 min. During the MVC testing, 10 stimuli (TS) at a 10-ms interval (100 Hz) were superimposed. The TS was used to assess the central activation ratio (CAR) of the quadriceps muscle [4, 5]. Six series of 60-s MVC were performed every 3 min (Figure 1); during the first session, these were performed at SL, while during the second session after a 3–5-day break, they were performed with the other leg and at LL (selection of right or left leg for each session was randomized). The sequence of each series was started by achieving MVC as fast as possible. This was the MVC value at the beginning of each series. After each series of 60-s MVC, the quadriceps muscle was relaxed and the MVC test (one trial as fast as possible with superimposed stimuli) was performed. MVC was performed once in all cases. Immediately afterwards, 1 Hz and 100 Hz ES of the muscle was delivered; 100 Hz stimulation lasted for 1 s. At 3, 10, and 30 min after HVHIE, muscle testing was repeated. During the 60-s MVC, we calculated the knee-extension torque integral every 10 s for the entire 60-s period. The maximal rate of force development during MVC (RFDv) and 100 Hz-induced ES (RFDs) were calculated.

EMG recording. EMG activity was recorded from the vastus lateralis of each leg. The skin was shaved and cleaned with alcohol to reduce the impedance between the skin and the electrode to less than 5 kΩ. EMG signals were obtained using two silver chloride electrodes (26 mm diameter; centre-to-centre distance 2.5 cm) (FIAB, Italy) attached to the bellies of the muscles. The common reference electrodes were placed under the knee caps. EMG signals were amplified (gain = 1000), band-pass filtered (10 Hz–5 kHz), digitized online (sampling rate 2 kHz), and recorded simultaneously using AcqKnowledge 4.1 software (BIOPAC Systems Inc., Goleta, CA, USA). EMG signals, such as the root mean square (EMGrms) in V and median frequency (MedF) in Hz, were extracted from a 1-s epoch coinciding with the 1-s force interval of the steady-state MVC. In addition, EMGrms was calculated for the first 50 and 100 ms during MVC.

Measurement of lactate. The concentration of lactate in capillary blood was measured using a blood lactate meter (Lactate Pro2; ARKRAY, The Netherlands). Lactate was measured before and 3 min and 30 min after HVHIE.

Saliva samples. Samples of saliva and blood were collected at three time points: before, 5 min after, and 30 min after the completion of six series of 60-s MVC (HVHIE). A minimum of 1 mL of saliva was collected into special tubes (IBL SaliCap, Germany). Samples were stored at –24°C for later analysis. The concentration of free cortisol in saliva was measured using an enzyme-linked immunosorbent assay (ELISA) and a Gemini analyser (Stratec Biomedical GmbH, Birkenfeld, Germany).

HR and HR variability (HRV) testing. Kubios HRV software (Department of Applied Physics, University of Kuopio, Finland) was used for HRV analysis [20]. We calculated the HR and the root mean square successive difference of interval (RMSSD) in ms. We judged the increase in stress based on the decrease in RMSSD [21]. HR and HRV were recorded and measured at 10 min before and 30 min after HVHIE. Additionally, these indices were recorded and measured during HVHIE.

Statistical analyses

Data are reported as means SD. The data were tested for normality using the Kolmogorov–Smirnov test, and all were found to be normally distributed. Two-way analysis of variance (ANOVA) for repeated measures (general linear mixed model) was used to determine the effect of six series of 60-s MVC (the effect of HVHIE) (six levels) and the effect of muscle length (SL vs. LL) on different parameters of skeletal muscle performance and EMG (both factors are within-subjects factors). Changes in HR and HRV and the concentrations of cortisol and lactate were also analysed using mixed model two-way ANOVA for repeated measures with time (three levels) and trial (SL vs. LL) (both factors are within-subjects factors). F value and the partial squared (η^2) were estimated as measures of the experimental trial effect size. Mauchly's test was implemented to check the assumption of sphericity, and if the assumption of sphericity was not met, a Greenhouse–Geisser correction was applied to the data. Post hoc testing was performed if significant effects were found via application of the paired t test using Bonferroni correction for multiple comparisons. For all tests, statistical significance was defined as $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics software (v. 22; IBM Corp., Armonk, NY, USA).

Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the local Ethics Committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

RESULTS

MVC, MVC-10 s integral, RFDv of MVC, P100, and RFDs of P100 were significantly higher and median frequency was lower at LL than at SL ($p < 0.01$) (Table 1).

Figure 2 shows the change in MVC-10 s integral over the 6 series at SL (A) and LL (B). MVC-10 integrals decreased significantly in all series at both SL and LL ($p < 0.001$). In the first two series, the force integral was larger at LL than at SL ($p < 0.01$) (Figure 2C). From the fourth to the sixth series, the force integral remained unchanged at both SL and LL. The change in 60-s MVC integral was significant ($F = 86.6$, $p < 0.001$, $\eta^2 = 0.76$) and depended on muscle length (decreased more for LL) ($F = 9.1$, $p < 0.001$, $\eta^2 = 0.26$). During the first four series, fatigue (decrease in MVC-10 s integral) was greater at LL than at SL ($p < 0.01$), although the change in fatigue index during HVHIE did not depend on muscle length ($F = 2.02$, $p = 0.137$, $\eta^2 = 0.075$) (Figure 2D).

Immediately after each MVC-60 series MVC and RFDv decreased more during HVHIE at LL than at SL (interaction effect of HVHIE and LL vs. SL, $F = 6.8$, $p < 0.001$, $\eta^2 = 0.22$ and $F = 3.4$, $p < 0.01$, $\eta^2 = 0.12$, respectively) (Figure 3A and B), while CAR remained unchanged in both cases ($F = 1.1$, $p = 0.37$, $\eta^2 = 0.04$) (Figure 3C). Immediately after each MVC-60 series RFDv decreased more than MVC during HVHIE at both LL and SL ($p < 0.01$). Neither MVC nor RFDv recovered within 30 min after exercise. MVC/P100 decreased during exercise in both LL and SL ($F = 6.7$, $p < 0.001$, $\eta^2 = 0.21$), but it did not depend on muscle length ($F = 0.99$, $p = 0.43$, $\eta^2 = 0.04$) (Figure 3D). However, during recovery, this ratio increased significantly in LL compared with baseline ($p < 0.01$). In addition, MVC/P100 after 30 min was significantly higher at LL than at SL ($p < 0.01$). This shows that central fatigue disappeared faster than peripheral fatigue.

MVC EMGrms (the effect of HVHIE: $F = 3.8$, $p = 0.06$, $\eta^2 = 0.13$) and median frequency ($F = 3.8$, $p = 0.09$, $\eta^2 = 0.13$) did not change significantly during exercise at either SL or LL (the effect of HVHIE x muscle length was not significant), but EMGrms during the first 50 ms (the effect of HVHIE: $F = 4.5$, $p < 0.001$, $\eta^2 = 0.154$) and 100 ms of MVC (the effect of HVHIE: $F = 4.5$, $p < 0.001$, $\eta^2 = 0.154$) decreased at both SL and LL and was independent of angle (the effect of HVHIE x muscle length for 50 ms and 100 ms, respectively: $F = 0.95$, $p = 0.43$, $\eta^2 = 0.03$; $F = 1.4$, $p = 0.21$, $\eta^2 = 0.05$) (Figure 4).

P1 (the effect of HVHIE: $F = 88.8$, $p < 0.001$, $\eta^2 = 0.78$) and P100 (the effect of HVHIE: $F = 215.8$, $p < 0.001$, $\eta^2 = 0.89$) decreased at both SL and LL, but to a greater extent at LL than at

TABLE 1. Baseline average values (± standard deviation) of motor performance. MVC – maximal voluntary contraction torque; RFDv and RFDs– rate of force development during MVC and P100 respectively; CAR – central activation ratio; MedF – median frequency of electromyography (EMG); MVCrms – root mean square of EMG during MVC; 50 ms and 100 ms MVCrms – root mean square of EMG during first 50 and 100 ms of MVC respectively; PI and P100 – electrostimulation induced torque at 1 Hz and 100 Hz respectively.

	Short length	Long length
MVC (Nm)	242.1 ± 51.7	303.1 ± 61.4#
RFDv of MVC (Nm/s)	2486.8 ± 670.9	3119.9 ± 588.8#
MVC 10 s integral (Nmxs)	1969.8 ± 427.8	2537.9 ± 466.7#
CAR (%)	98.8 ± 0.9	98.9 ± 0.8
MedF (Hz)	72.1 ± 13.6	65.0 ± 5.2#
MVCrms (V)	0.50 ± 0.23	0.46 ± 0.25
50 ms MVCrms (V)	0.41 ± 0.21	0.35 ± 0.19
100 ms MVCrms (V)	0.54 ± 0.28	0.53 ± 0.2
50 ms MVCrms/MVCrms (%)	90.1 ± 31.1	80.4 ± 36.2
100 ms MVCrms/MVCrms (%)	123.4 ± 35.5	124.9 ± 37.7
P1 (Nm)	21.1 ± 8.0	24.5 ± 4.4
P100 (Nm)	197.5 ± 36.2	226.2 ± 61.8#
P1/P100 (%)	10.6 ± 2.3	10.8 ± 2.9
RFDs of P100 (Nm/s)	2664.0 ± 448.1	2730.2 ± 599.7#
MVC/P100	1.22 ± 0.34	1.35 ± 0.25

– $p < 0.01$ compared to short length.

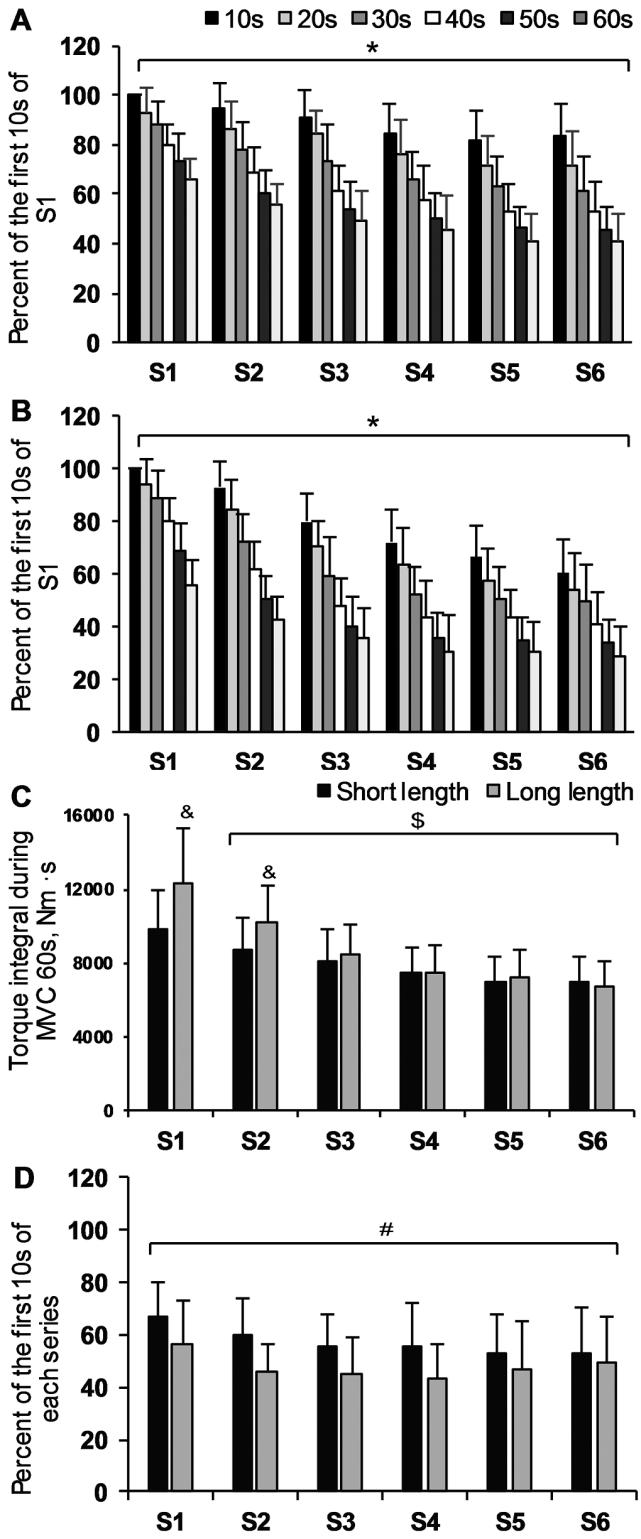

FIG. 2. Changes in torque integral during each 10 s of 6 series (S1, S2, S3, S4, S5, S6) of MVC-60 s at short (A) and long (B) quadriceps muscle lengths and changes in torque integral during 60 s of MVC (C) and decrease in torque integral of 10 s of each 6 series (D) (S1, S2, S3, S4, S5, S6) of MVC-60 (e.g. MVC-50–60 s/MVC-10 s x 100 percent in each series) at short and long quadriceps muscle lengths. Data are presented as mean ± SD. * – $p < 0.01$ compared to the first 10 s of the first series of MVC-60 s; # – $p < 0.01$ 10 s torque integral significant decrease during MVC-60 s; & – $p < 0.01$ compared to the short length; $ – $p < 0.01$ compared to the first series of MVC-60 s.

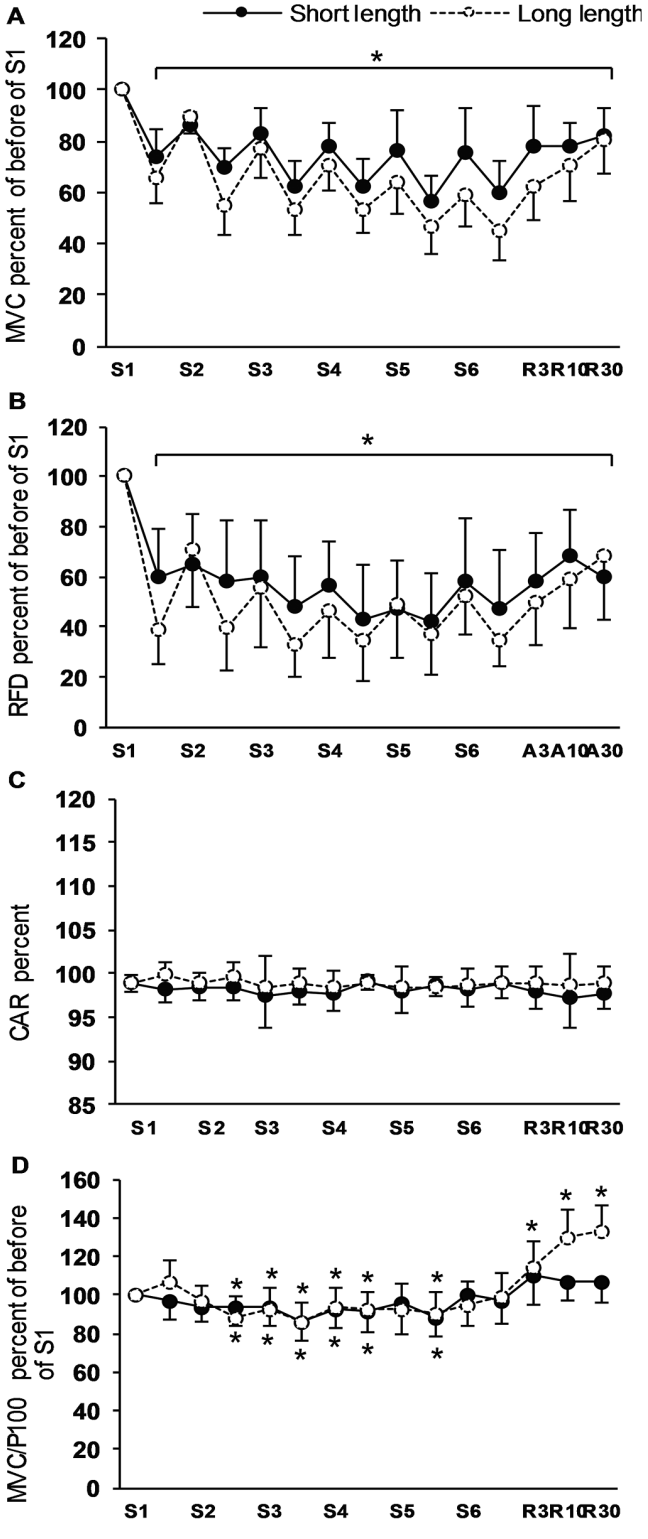

FIG. 3. Changes in maximal voluntary contraction torque (MVC) (A), rate of force development (RFD) during MVC (B), central activation ratio (CAR) (C) and MVC/P100 before and after each 6 series (S1, S2, S3, S4, S5, S6) of MVC-60 s at short and long quadriceps muscle lengths. MVC, RFD, CAR and MVC/P100 values are given at the beginning of each series and immediately after the end of each series. P100 – electrostimulation induced torque at 100 Hz. R3, R10 and R30 – recovery after 3, 10 and 30 min after exercise respectively. Data are presented as mean ± SD. * – $p < 0.01$ compared to before of S1.

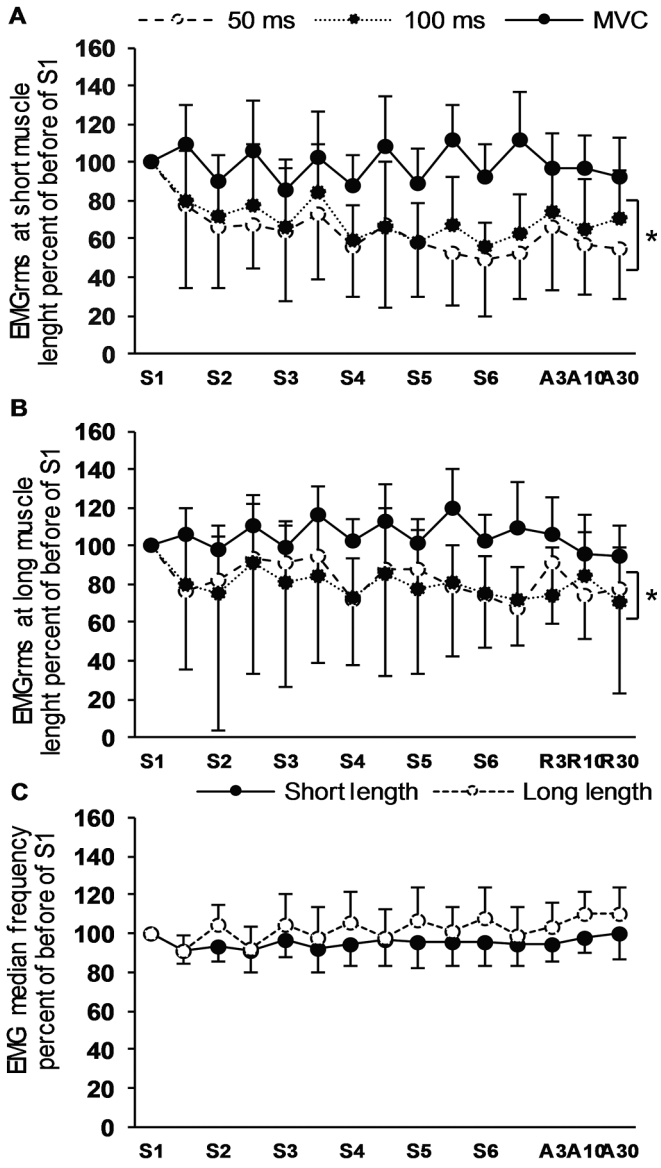

FIG. 4. Changes in EMGrms (A and B) and median frequency of EMG (C) at the beginning of each series and after each 6 series (S1, S2, S3, S4, S5, S6) of MVC-60 at short and long quadriceps muscle lengths (100 percent is the beginning of S1). R3, R10 and R30 – recovery after 3, 10 and 30 min after exercise respectively. Data are presented as mean ± SD.
* – $p < 0.01$ compared to before of S1.

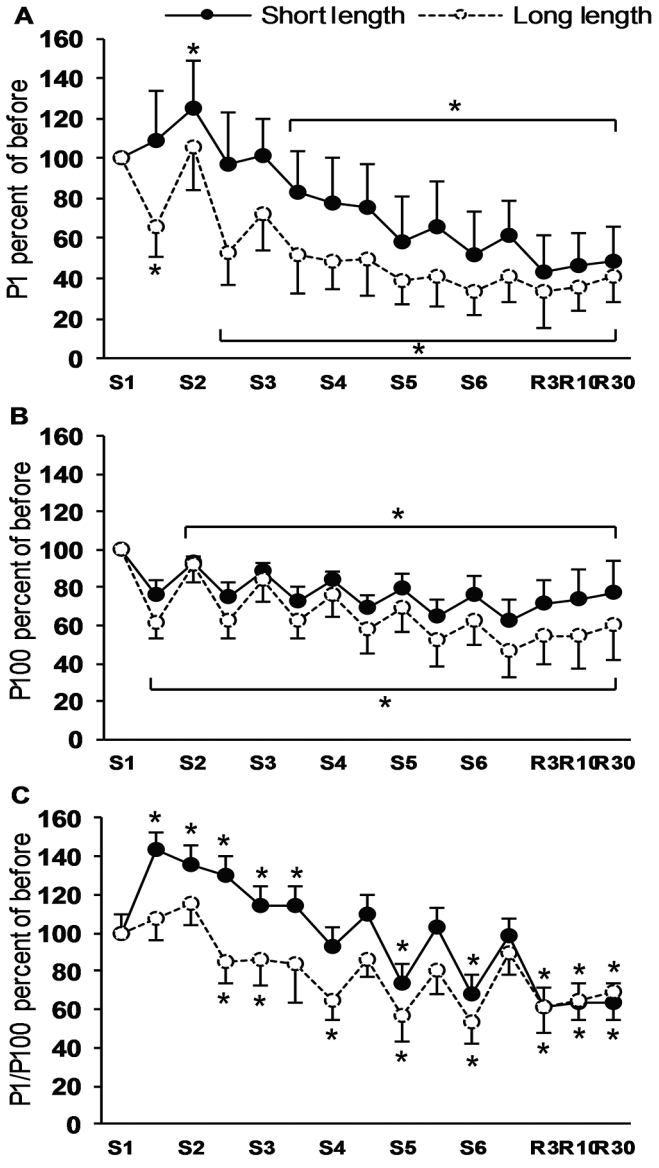

FIG. 5. Changes in electrically induced torque at 1 Hz (A), 100 Hz (B) and P1/P100 before and after each 6 series (S1, S2, S3, S4, S5, S6) of MVC-60 s at short and long quadriceps muscle lengths. R3, R10 and R30 – recovery after 3, 10 and 30 min after exercise respectively. Data are presented as mean ± SD.
* – $p < 0.01$ compared to before of S1.

SL (the effect of HVHIE x muscle length for P1 and P100, respectively: $F = 15.1$, $p < 0.001$, $\eta^2 = 0.38$; $F = 6.5$, $p < 0.001$, $\eta^2 = 0.21$) (Figure 5). In addition, for SL, post-tetanic potentiation occurred at the onset of exercise because P1 increased ($p < 0.001$). In both SL and LL, low-frequency fatigue occurred after exercise (the effect of HVHIE: $F = 80.1$, $p < 0.001$, $\eta^2 = 0.68$; the effect of HVHIE x muscle length: $F = 1.95$, $p = 0.13$, $\eta^2 = 0.06$).

P100 RFDs decreased during exercise at both LL and SL (the effect of HVHIE: $F = 84.6$, $p < 0.001$, $\eta^2 = 0.77$), but to a greater extent at LL than at SL (the effect of HVHIE x muscle length: $F = 7.8$, $p < 0.001$, $\eta^2 = 0.24$) (Figure 6). RFDv MVC/RFDs P100 also decreased during exercise at both SL and LL (the effect of HVHIE: $F = 7.8$, $p < 0.001$, $\eta^2 = 0.24$) and did not depend on muscle length (the effect of HVHIE x muscle length: $F = 7.8$, $p < 0.001$, $\eta^2 = 0.24$), but it increased at LL during recovery and was greater than the baseline value after 30 min ($p < 0.01$).

After exercise, lactate increased significantly and to a greater extent at LL than at SL (the effect of HVHIE x muscle length: $F = 7.9$,

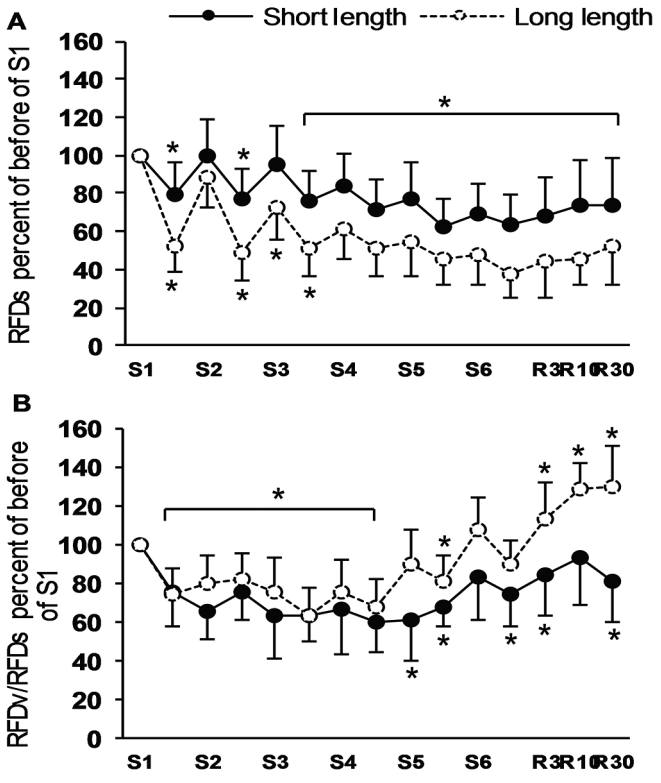

FIG. 6. Changes in rate of force development during electrically induced 100 Hz stimulation (RFDs) (A) and RFDv/RFDs before and after each 6 series (S1, S2, S3, S4, S5, S6) of MVC-60 s at short and long quadriceps muscle lengths. RFDv – rate of force development during maximal voluntary contraction. R3, R10 and R30 – recovery after 3, 10 and 30 min after exercise. Data are presented as mean ± SD.
* – $p < 0.01$ compared to before of S1.

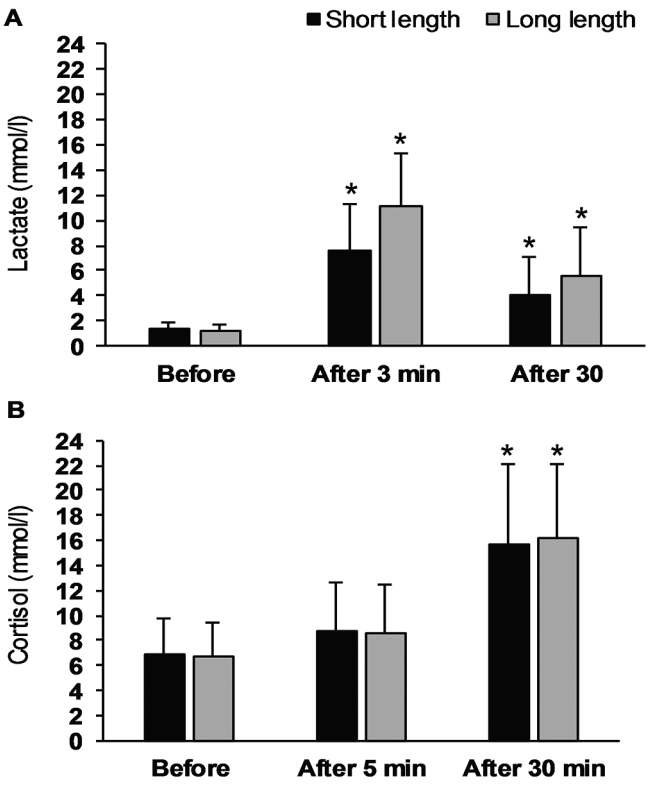

FIG. 7. Changes in lactate (A) and cortisol (B) concentration after 6 series of MVC-60 at short and long quadriceps muscle lengths. Data are presented as mean ± SD.
* – $p < 0.01$ compared to before.

$p < 0.001$, $\eta^2 = 0.23$) (Figure 7A). For both SL and LL, cortisol levels increased significantly only at 30 min after exercise ($p < 0.001$) (the effect of HVHIE: $F = 5.9$, $p < 0.001$, $\eta^2 = 0.21$) (interaction effect was not significant) (Figure 7B).

During exercise, HR increased equally at both LL and SL of HVHIE ($p < 0.001$) (respectively from 70.8 ± 9.1 and 71.5 ± 7.5 beats/min before to 131.8 ± 16.6 and 128.5 ± 13.8 beats/min during HVHIE). RMSSD decreased equally at both LL and SL of HVHIE ($p < 0.001$) (respectively from 44.8 ± 6.1 and 40.4 ± 5.3 ms before to 9.2 ± 5.4 and 9.5 ± 4.1 ms during HVHIE. HR and RMSSD did not recover to the baseline value within 30 min ($p < 0.001$ compared to before/baseline).

DISCUSSION

The main novelty of our study was the finding that HVHIE performed with one leg caused great stress to the body (first of all to the neuromuscular system) (cortisol levels approximately doubled, RMSSD decreased to 25%, lactate increased to 8–11 mmol/L, voluntary and 100 Hz stimulation-induced force [recorded immediately after the end of the HVHIE] decreased to 45% [at LL] and 60% [at SL]), although CAR remained unchanged after exercise at both SL and LL. This suggests that fatigue was localized more in the muscles than in the CNS during this exercise. It was rather unexpected that central fatigue did not start to increase during such heavy exercise, because our previous studies showed that CAR decreased even after lighter exercise, e.g., after 100 intermittent isometric contractions at an intensity of 50% of MVC [22], at the end of 2-min MVC [4], after 12 series of 5-s veloergometric exercise at maximal intensity [6, 18], and after 100 [23] or 200 drop jumps at maximal intensity [18]. In fact, cortisol levels after HVHIE were higher than those after drop jumps [18] or after veloergometric exercise at maximal intensity [6]; after 100 intermittent isometric contractions, cortisol levels even decreased [22]. We expected that the six 60-s series of MVC at both SL and LL would result in severe central fatigue because of high levels of peripheral afferent signals. Prolonged exercise activates III/IV afferents, which send signals to the CNS and this might "protect"

muscles from further exhaustion [15, 17]. We do not know any reason why CAR did not decrease immediately after such intense exercise. This definitely needs to be investigated in other studies in the future.

The second hypothesis (and the second main finding) of our study was not confirmed, because low-frequency fatigue (decrease in P1P/100) after HVHIE at LL was not higher than after exercise at SL (Figure 5C). Our previous research shows that prolonged low-frequency fatigue is often associated with skeletal muscle mechanical damage [5, 23]. After HVHIE at LL, lactate concentrations were higher than after HVHIE at SL. In addition, although MVC and P100 at LL decreased significantly, P1/P100 did not differ between SL and LL 30 min after exercise. This shows that low-frequency fatigue was not greater after exercise at LL. Therefore, there is no reason to believe that the muscle mechanical damage at LL could have been greater than the one at SL. However, the data in our studies do not allow us to determine whether there was muscle mechanical damage at all because we did not measure the concentration of creatine kinase, which could more accurately show the degree of mechanical muscle damage. [5, 23]. P1/P100 decreased to about 60% 30 min after exercise at both LL and SL. This is consistent with our previous findings that the P20/P100 decreased to 50–60% after a 3 × 30-s Wingate test, 2-min MVC isometric exercise, or after 100 drop jumps [5]. In addition, our previous studies showed that P10 decreased to 40% of the baseline value 30 min after a 6 × 30-s Wingate test [10], which is very similar to the present finding that P1 decreased to 40% (Figure 5A).

There is no doubt that the resultant (net) muscle contraction force (MVC, P1, and P100), RFDv during MVC, and P100 during and after HVHIE are dependent on many coexisting factors, e.g., post-activation potentiation, tetanic maximal force potentiation, low-frequency fatigue, metabolic-related fatigue, post-contractile depression, and exercise-induced muscle damage [3, 5, 24]. It is often quite difficult to distinguish the effects of these factors. However, the observation that during the first series of HVHIE, P1 did not decrease, and at SL it even increased, indicated that post-tetanic potentiation was clearly present at the onset of exercise and within 3 min of recovery. This is consistent with the previous findings in our studies that P1 change is dependent on the interaction of fatigue and post-tetanic potentiation, i.e. when fatigue disappears after exercise but post-tetanic potentiation remains, P1 even increases during recovery [3, 5, 24]. In the case of this study, after 4–6 series, the onset of post-tetanic potentiation can no longer compensate for high muscle fatigue, and therefore P1 decreased even more and did not recover between the series.

Our third main finding was that after HVHIE, the RFDv of MVC declined more than the MVC itself. This is consistent with other reports that the RFDv was lower in fatigue than in MVC [25, 26]. We believe that during HVHIE in our study, there was an increase in the impairment of the ability of the CNS to maximally activate the muscle in the first milliseconds of the contraction. This is demonstrated by the decrease in EMG at 50 and 100 ms (Figure 4). In addition, we observed that the RFDv of MVC/RFDs of P100 significantly decreased during exercise (Figure 6B). Undoubtedly, the decrease in RFDv may have been influenced not only by voluntary activation mechanisms but also by involuntary/reflexive mechanisms, which can suppress many supraspinal and spinal mechanisms responsible for RFDv, for example, for the recruitment of motor units and the frequency of impulses. There is no doubt that the evaluation of RFDv in human skeletal muscle is a complex task that is influenced by numerous distinct methodological factors, including the mode of contraction, type of instruction, method used to quantify RFD, devices used for force/torque recording, and ambient temperature [27, 28, 29, 30].

We chose spectral EMG analysis because most of the evidence suggests that the frequency domain of the EMG signal contains some information regarding motor-unit firing rates [31], and the size of the EMGrms indicates the muscle neural activation level [31, 32]. In our study, EMGrms and the median frequency of EMG during MVC did not change significantly at either LL or SL (Figure 4).

The main limitation of these studies is that we studied only young men, so the findings of our studies cannot be directly applied to young women, older men and women, or to boys and girls.

CONCLUSIONS

In conclusion, when HVHIE is performed with one leg in isometric mode, the body experiences high levels of stress (RMSSD decreased, cortisol increased after HVHIE equally at SL and LL; La increased more while exercising at LL), voluntary and ES-induced muscle force significantly decrease, but muscle central activation does not decrease during MVC.

Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

1. Zinner C, Morales-Alamo D, Ørtenblad N, Larsen FJ, Schiffer TA, Willis SJ. The physiological mechanisms of performance enhancement with sprint interval training differ between the upper and lower extremities in humans. Front Physiol. 2016; 7:426.
2. Bishop DJ, Botella J, Genders AJ, Lee MJ, Saner NJ, Kuang J. High-intensity exercise and mitochondrial biogenesis: current controversies and future research directions. Physiology (Bethesda). 2019; 34(1):56–70.
3. Skurvydas A, Zachovajevas P. Is post-tetanic potentiation, low frequency fatigue (LFF) and pre-contractile depression (PCD) coexistent in intermittent isometric exercises of maximal intensity? Acta Physiol Scand. 1998; 164:127–133.
4. Brazaitis M, Skurvydas A, Pukenas K, Daniuseviciute L, Mickeviciene D,

Solianik R. The effect of temperature on amount and structure of motor variability during 2-minute maximum voluntary contraction. Muscle Nerve. 2012; 46:799–809.
5. Skurvydas A, Mamkus G, Kamandulis S, Dudoniene V, Valanciene D, Westerblad H. Mechanisms of force depression caused by different types of physical exercise studied by direct electrical stimulation of human quadriceps muscle. Eur J Appl Physiol. 2016; 116:2215–2224.
6. Skurvydas A, Verbickas V, Eimantas N, Baranauskiene N, Cernych M, Skrodeniene E et al. Psychological and physiological biomarkers of neuromuscular fatigue after two bouts of sprint interval exercise. Front Psychol. 2017; 8:2282.
7. Carroll TJ, Taylor JL, Gandevia SC. Recovery of central and peripheral neuromuscular fatigue after exercise. J Appl Physiol. 2017; 122(5):1068–1076.
8. Cheng AJ, Place N, Westerblad H. Molecular basis for exercise-induced fatigue: the importance of strictly controlled cellular Ca2+ handling. Cold Spring Harb Perspect Med. 2018;1:8(2).
9. Allen D, Lamb G, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008; 88:287–332.
10. Place N, Ivarsson N, Venckunas T, Neyroud D, Brazaitis M, Cheng AJ et al. Ryanodine receptor fragmentation and sarcoplasmic reticulum Ca2+ leak after one session of high-intensity interval exercise. Proc Natl Acad Sci. USA 2015; 112:15492–15497.
11. Debold EP, Fitts RH, Sundberg CW, Nosek TM. Muscle fatigue from the perspective of a single crossbridge. Med Sci Sports Exerc. 2016; 48(11):2270–2280.
12. Allen TJ, Jones T, Tsay A, Morgan DL, Proske U. Muscle damage produced by isometric contractions in human elbow flexors. J Appl Physiol. 2018; 124(2):388–399.
13. Taylor JL, Todd G, Gandevia SC. Evidence for a supraspinal contribution to human muscle fatigue. Clin Exp Pharmacol Physiol. 2006; 33(4):400–405.
14. Sidhu SK, Weavil JC, Thurston TS, Rosenberger D, Jessop JE, Wang E et al. Fatigue-related group III/IV muscle afferent feedback facilitates intracortical inhibition during locomotor exercise. J Physiol. 2018; 596(19):4789–4801.
15. Amann M, Dempsey JA. Ensemble input of group III/IV muscle afferents to CNS: a limiting factor of central motor drive during endurance exercise from normoxia to moderate hypoxia. Adv Exp Med Biol. 2016; 903:325–342.
16. Hureau TJ, Ducrocq GP, Blain GM. Peripheral and central fatigue development during all-out repeated cycling sprints. Med Sci Sports Exerc. 2016; 48(3):391–401.
17. Hureau TJ, Romer LM, Amann M. The 'sensory tolerance limit': a hypothetical construct determining exercise performance? Eur J Sport Sci. 2018; 18(1):13–24.
18. Verbickas V, Baranauskiene N, Eimantas N, Kamandulis S, Rutkauskas S, Satkunskiene D et al. Effect of sprint cycling and stretch-shortening cycle exercises on the neuromuscular, immune and stress indicators in young men. J Physiol Pharmacol. 2017; Feb;68(1):125–132.
19. Fiorenza M, Hostrup M, Gunnarsson TP, Shirai Y, Schena F, Iaia FM et al. Neuromuscular fatigue and metabolism during high-intensity intermittent exercise. Med Sci Sports Exerc. 2019; Feb 26. doi: 10.1249/ MSS.0000000000001959. [Epub ahead of print].
20. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV-heart rate variability analysis software. Comput Methods Programs Biomed. 2014;113(1):210–220.
21. Acharya UR, Joseph KP, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Biol Eng Comput. 2006; 44(12):1031–1051.
22. Kyguoliene L, Skurvydas A, Eimantas N, Baranauskienė N, Mickeviciene D, Urboniene D et al. Effect of constant, predictable, and unpredictable motor tasks on motor performance and blood markers of stress. Exp Brain Res. 2017; 235(5):1323–1336. .
23. Skurvydas A, Brazaitis M, Venckunas T, Kamandulis S. Predictive value of strength loss as an indicator of muscle damage across multiple drop jumps. Appl Physiol Nutr Metab. 2011; 36(3):353–360.
24. Skurvydas A, Jurgelaitiene G, Kamandulis S, Mickeviciene D, Brazaitis M, Valanciene D et al. What are the best isometric exercises of muscle potentiation? Eur J Appl Physiol. 2019 Apr; 119(4):1029–1039.
25. Morel B, Rouffet DM, Saboul D, Rota S, Clémençon M, Hautier CA. Peak torque and rate of torque development influence on repeated maximal exercise performance: contractile and neural contributions. PLoS One 2015; 10(4):e0119719.
26. Grazioli R, Lopez P, Andersen LL, Machado CLF, Pinto MD, Cadore EL et al. Hamstring rate of torque development is more affected than maximal voluntary contraction after a professional soccer match. Eur J Sport Sci. 2019 ;1:1–6. doi :10.1080/17461391.2019.1620863.
27. Folland JP, Buckthorpe MW, Hannah R. Human capacity for explosive force production: neural and contractile determinants. Scand J Med Sci Sports. 2014; 24(6):894–906.
28. Maffiuletti NA, Aagaard P, Blazevich AJ, Folland J, Tillin N, Duchateau J. Rate of force development: Physiological and methodological considerations. Eur J Appl Physiol. 2016; 116(6):1091–1116.
29. Rodríguez-Rosell D, Pareja-Blanco F, Aagaard P, González-Badillo JJ. Physiological and methodological aspects of rate of force development assessment in human skeletal muscle. Clin Physiol Funct Imaging. 2017. doi: 10.1111/cpf.12495.
30. Del Vecchio A, Falla D, Felici F, Farina D. The relative strength of common synaptic input to motor neurons is not a determinant of the maximal rate of force development in humans. J Appl Physiol. 2019;127(1):205–214.
31. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG: an update. J Appl Physiol. 2004; 117(11):1215–1230. .
32. Staudenmann D, Roeleveld K, Stegeman DF, van Dieën JH. Methodological aspects of SEMG recordings for force estimation--a tutorial and review. J Electromyogr Kinesiol. 2010; 20(3):375–387.