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Alterations in Muscle Contractile Properties, Structure, and Function during 10-d Bed Rest, Postrecovery, and after COVID-19 Lockdown

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ABSTRACT

ŠIMUNIČ B., F. SARTO, M. V. FRANCHI, E. MONTI, R. PIŠOT, and M. V. NARICI. Alterations in Muscle Contractile Properties, Structure, and Function during 10-d Bed Rest, Postrecovery, and after COVID-19 Lockdown. *Med. Sci. Sports Exerc.,* Vol. 58, No. 8, pp. 1674–1686, 2026. **Purpose:** Tensiomyography detects early hallmarks of bed-rest-induced atrophy. We aimed to identify early tensiomyography alterations in six muscles during a 10-d bed rest (BR10), followed by a 30-d recovery period (R+30), and to compare these changes with those observed after the 50-d COVID-19 lockdown in 10 healthy males (22.9 ± 5.0 yr). **Methods:** Tensiomyography and muscle thickness (assessed by ultrasound imaging) were assessed at baseline, during bed rest, at BR10, R+30, and after the COVID-19 lockdown. Additional assessments included vertical jump performance (force plate), body composition (bioimpedance), physical activity (Global Physical Activity Questionnaire), and lower back pain (VAS). **Results:** By BR10, participants experienced a 1.9% body mass loss, a 3.8% increase in fat mass, and a 2.4% reduction in muscle mass. Although anthropometric parameters returned to baseline by R+30, fat mass increased again by 3.9% postlockdown. Jump height declined by 10.1% at BR10, returned to baseline at R+30, and remained stable thereafter. Muscle thickness decreased by 7.0% in the vastus lateralis at BR10 and by 10.1% postlockdown. Tensiomyographic amplitude increased in five leg muscles before BR10, recovered at R+30 and increased again after postlockdown, whereas a reduction was observed in the erector spinae within the first 48 h, coinciding with reported lower back pain. In the erector spinae, both delay and contraction time decreased during BR10 and postlockdown, whereas contraction time of the biceps femoris increased before BR10 and remained elevated postlockdown. **Conclusions:** The observed post COVID-19 lockdown alterations in tensiomyographic parameters followed the same trend as those induced by bed rest, albeit with smaller magnitude. These findings support the utility of TMG as a sensitive and noninvasive method for monitoring muscle responses across different disuse paradigms. **Key Words:** ATROPHY, DISUSE, SKELETAL MUSCLE, TENSIOMYOGRAPHY, ULTRASOUND

Changes in muscle size and volume (1–4) and in muscle architecture (5–7) of different lower limb muscles have been found after exposure to unloading periods obtained by experimental bed rest. Besides muscle structural changes, reductions in maximal force capacity (1,8), explosive power (1), activation capacity (8), and rate of force development (9) have been frequently reported after periods of bed rest. Several studies have demonstrated that periods of physical inactivity in older adults exacerbate the reductions in muscle mass and function in younger individuals (10). Older participants also showed reduced recovery capacity of neuromuscular function as compared to younger participants after physical retraining that followed disuse (1,11–14). Thus, there is a substantial need to validate methods to detect early stages of muscle atrophy and weakness-related processes.

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Impaired muscular structure and function increase the risk of fractures, owing to falling (15), and worsen the general health conditions of older individuals, including protein and glucose metabolism (16), cardiovascular function (17), and cognitive capacity (18) impairment. It follows that pairing aging with physical inactivity consequences has great clinical relevance, as the combination of these two conditions is common among aged individuals.

Tensiomyography (TMG) measures muscle belly displacement during twitch-evoked isometric contraction and is an increasingly recognized method to assess muscle contractile properties *in vivo* (19). It has previously been used to evaluate muscle disuse in several bed rest studies (5,7,20). Previously, in a 35-d horizontal bed rest study, we reported an increased TMG amplitude (Dm) in leg gastrocnemius medialis (GM), vastus medialis (VM), and long head of biceps femoris (BFlh) but not in upper body biceps brachii (BB) in younger adults (20). Similarly, in a next 35-d 6° head-down tilt (HDT) bed rest study, also performed in younger adults, we reported similar increase in Dm in all three studied leg muscles VM and BFlh (7). However, when assessing TMG and muscle architecture daily, we found that Dm increases preceded changes in muscle architecture by 1–6 d, in each assessed muscle (7), suggesting that TMG assessment of contractile properties may be more sensitive than conventional muscle morphological measurements. In the same study, we also carried out a 30-d recovery period and reported full recovery of Dm and muscle architecture parameters but not in TMG contraction time (Tc) that was elevated in BFlh (but not in other muscles) at the end of the bed rest period. In our previous report from this study, we examined BFlh adaptations after a 10-d horizontal bed rest and documented a reduction in muscle cross-sectional area after 10 d of bed rest, a shortening of fascicle length after 6 d of bed rest, and no measurable change in pennation angle. However, TMG Tc and Dm increased after 10 d and 2 d of bed rest, respectively (5). Furthermore, we have found a correlation between increased BFlh Dm and diminished fascicle length, cross-sectional area, volume (5), as well as between increased GM Dm increase and thickness decrease (20). TMG was used to assess main leg muscles after bed rest (5,7,20), and there are no data for erector spinae (ES) muscle, although 70% of astronauts and 67% of bed rest participants report low back pain (LBP) during early days of missions or bed rest, respectively (21).

This secondary report expands our previous publication, which focused exclusively on hamstring adaptations in the same cohort of 10 young men after 10 d of bed rest (5). Specifically, the present study 1) assesses immediate and early changes in contractile properties as early as 3 h after bed rest onset; 2) introduces a TMG parameter not previously applied in disuse studies, namely delay time (Td); 3) examines adaptations in the ES and their associations with LBP, which is commonly reported in astronauts and microgravity analogues, such as a bed rest (21), alongside changes in five lower limb muscles; and 4) compares the deterioration induced by bed rest with the milder reduction in physical activity associated with the subsequent 2-month government-mandated COVID-19 lockdown, given that the bed-rest campaign was conducted in September 2019. Although the project was originally designed to investigate early muscle adaptations to simulated microgravity exposure, no previous study has directly compared the consequences of bed rest with those of public health measures restricting movement within a longitudinal study design. During the COVID-19 pandemic, numerous public measures were implemented to limit mobility, and their effects were widely reported as reductions in physical activity, increases in sedentary behavior, and alterations in eating habits (22). The present results may therefore provide valuable insight into the clinical applicability of TMG and enhance our understanding of the physiological consequences of movement restrictions imposed by public health interventions, although the study is largely underpowered for firm conclusions.

Based on the assumption that 10 d of horizontal bed rest, in younger males, would lead to muscle atrophy and decreased muscle function, we hypothesized that TMG Dm would increase already during the bed rest period, but nonuniformly between different leg muscles. Whereas in ES muscle TMG Dm would decrease owing to increased LBP in the initial days of bed rest. We also hypothesized that TMG Tc would remain unchanged. We also expected that the COVID-19 lockdown would affect neuromuscular function similarly to bed rest, however, with a lower magnitude.

MATERIALS AND METHODS

Participants

Participants were described in our previous publication (23); however, we provide a summary here, for completeness and because the present secondary analysis includes the same participants with additional outcomes and time points. Ninety-three young males applied to participate in this study. Forty-six of them were invited to the interviews and 16 passed all inclusion/exclusion criteria and medical examination. To reach a statistical power >0.85, an 0.05 alpha was chosen, an expected effect size (Cohen's $f = 1.5$) and the correlation between time points ($r = 0.6$) were estimated from previous studies of comparable bed rest duration for Dm (5,7). Based on these parameters, a power analysis performed using G*Power indicated that a minimum of four participants would be sufficient. The 10 most homogenous participants were selected, with two additional volunteers as reserves. Ten healthy males (aged 22.9 ± 5.0 yr, body mass 77.5 ± 10 kg, height 181.2 ± 3.9 cm, body mass index: 23.6 ± 2.5 kg·m^{-2}) took part in a 10 d horizontal bed rest study. All participants passed kinesiological and medical examination, physical activity questionnaire (GPAQ), body composition analysis, resting and exercise electrocardiography with blood pressure assessment, medical questionnaires, functional movement assessment, and a nutrition interview. Inclusion criteria were 20–32 yr; 21 < body mass index < 28 kg·m^{-2}; moderate-to-vigorous physical activity <90 daily minutes; all-eating diet; normal electrocardiography; and blood pressure. Exclusion criteria were international level athletes; regular alcohol consumption; ferromagnetic implants; history of deep vein thrombosis with D-dimer >500 μg·L^{-1}; acute or chronic skeletal, neuromuscular, metabolic, and cardiovascular disease conditions; pulmonary embolism. None of the participants experienced any significant disease, and none was taking medications regularly or made use of any medication known to influence physical performance. This work was part of a larger project funded by the Italian Space Agency (MARSPRE2019, project no. DC-VUM-2017-006) aiming to detect early biomarkers of neuromuscular degeneration after short-term bed rest. Other neuromuscular data from this project were published in several studies (5,23–25).

Experimental Protocol

The present study was conducted in the General Hospital of Izola (Izola, Slovenia). The protocol included 3 d of familiarization with the study environment and diet during which baseline data were collected (BDC), data collection after 3 h of bed rest (BR3h) and after i-th day of bed rest (BRi; where i = 2, 4, 6, 8, 10) or after i-th day of recovery (R+i; where i = 2, 30) and data collection after the COVID-19 lockdown period (COVID-19).

The participants were housed in standard air-conditioned hospital rooms and were under constant visual surveillance with 24-h medical care, 24-h heart rate, and physical activity monitors. During the bed rest, the participants performed all daily activities in bed with no deviations from the horizontal lying position permitted, and without work out of any kind during the bed rest period. All participants received hospital meals three times a day and followed an individually controlled eucaloric diet during the whole hospital stay. Dietary energy requirement was designed for each subject multiplying resting energy expenditure by factors 1.2 and 1.4 in the bed rest and ambulatory periods, respectively (26). The macronutrient food content was set at 60% carbohydrates, 25% fat, and 15% proteins. The sleeping time was between 22:00 and 7:00 h.

The study was conducted in accordance with the declaration of Helsinki and was approved by The National Medical Ethics Committee of the Republic of Slovenia (No. 0120-123/2023/9). All participants were fully informed of the procedures and gave written informed consent. The bed rest was carried out in August and September 2019 in the Izola General Hospital (Izola, Slovenia) and assessment after the 2-month COVID-19 lockdown in May 2020 in the Laboratory of the Institute for Kinesiology Research at Science and Research Center Koper.

After being discharged from the hospital, participants were free to perform all daily tasks at will; however, they were required to follow the recovery plan. Recovery after the bed rest was not supervised by our personnel although personal trainers in local fitness clubs were hired and given a nonpersonalized recovery plan. Personal trainers assured us that all participants underwent a 30-d recovery, including three 60-min sessions weekly in local fitness clubs. The first recovery session was supervised by our research team, on the day of hospital release. The recovery plan consisted of 10 min of warm-up (treadmill or stationary bike), 20 min of circular training for balance and lower body strength (3 series × 20 repetitions: calf raises, knee extensions, knee flexions, or 60 s: balance board), 20 min of endurance workout (treadmill running or stationary biking at 5–7 Borg effort scale), and 10 min of stretching and cooldown.

To study changes after COVID-19 lockdown was not initially planned; however, we had access to our bed rest participants in COVID-19 era and decided to invite them for additional screening on May 12, 2020, 7 d after 50-d restriction from outside physical activity. The COVID-19 restrictions in Slovenia were valid until May 5, 2020, specifically: lockdown of schools from March 12, 2020; lockdown of physical activity, sports centers, and exiting homes from March 16, 2020; lockdown of travel from March 30, 2020 (27).

The following parameters were collected at all time points: anthropometric features, vertical jumping performance and, tensiomyography of six muscles. Ultrasonography assessment of four muscles was carried out every 2 d during bed rest, with data available also after the COVID-19 lockdown for one muscle.

Assessments

Anthropometry. Body height and mass were collected using a stadiometer and scale, respectively. Although whole body fat and lean mass were collected using bioimpedance analysis (BIA 101 Anniversary, Akern s.r.l., Pisa, Italy). Bioimpedance analysis was performed supine after at least 60-min rest.

Tensiomyography. The noninvasive TMG method was used to assess the contractile properties of six selected muscles: VL, VM, BFlh, GM, tibialis anterior (TA), and ES. TMG assessment of the VL was limited owing to the muscle biopsies performed on that muscle of both legs, with the corresponding analysis outcomes reported elsewhere (23). Therefore, we are presenting only data from the BDC and BR10 (just before and 10 d from the VL biopsy). Assessments of BFlh, GM, and ES were performed prone at rest and with a knee angle set at 5° flexion, whereas assessments of VL, VM, and TA were measured, whereas supine at rest and with a knee angle set at 30° flexion while ankle angle in neutral position. Foam pads were used for leg support to ensure rested muscles and joint angles. The oscillations of the belly muscle in response to an electrically induced isometric twitch were recorded at the skin surface using a sensitive digital displacement sensor (TMG-BMC Ltd., Ljubljana, Slovenia). The sensor was set perpendicular to the skin's normal plane above the belly muscle: at the midpoint of the line between the fibula head and the ischial tuberosity on the BFlh; at 30% of the femur length above the patella on the VL and 20% of the femur length above the patella on the medial side on the VM; at the height of the iliac crest on the ES (longissimus part); at 30% of the muscle length from the most distal part of the muscle on the GM; and 50% of the muscle length on the TA. In all muscles, the reference point was determined at the midmuscle width and marked daily with a dermatological pen during the bed rest period. The rounded (5-cm diameter) self-adhesive cathode and anode (Axelgaard, Aarhus, Denmark) were set 5 cm distally and 5 cm proximally, respectively, to the measuring point on all muscles assessed (28). Electrical stimulation was applied through a TMG-100 system electro stimulator (TMG-BMC Ltd., Ljubljana, Slovenia) with a pulse width of 1 ms and an initial amplitude of 30 mA. During each testing session, the amplitude was progressively increased by 20 mA increments until there was no further increase in the amplitude of the TMG response (Dm). Rest periods between stimuli of 10 s were given to avoid fatigue and potentiation. From two maximal twitch responses, the TMG parameters were calculated as follows: Td as the time from the electrical impulse to 10% Dm; Tc as the time from 10% to 90% Dm (29), and the average was taken for further analysis. Previously it was shown that the intra-rater reliability for Td, Tc and Dm was excellent in VL and BFlh muscle (Td: ICC > 0.89 and CV < 2.6%; Tc: ICC > 0.98 and CV < 4.9%; and Dm: ICC = 0.99 and CV < 4.7%) (30). The BFlh data were partially presented elsewhere (5) but are included here in full, with additional contractile parameters, time points, and statistical analyses.

Ultrasound imaging. Morphology of different muscles (VL, BFlh, TA, GM) was assessed by experienced examiner in vivo at rest by B-mode ultrasonography (Mylab 70; Esaote Biomedica, Genova, Italy), using a linear 4.7-cm probe. VL and BFlh scans were acquired in the longitudinal plane at 50% of the femur length, determined as the distance between the greater trochanter and the mid-patella point. TA and GM scans were acquired at 50% of the muscle length, determined after identifying the proximal and distal muscle insertions using ultrasound. During all image acquisitions, the operator maintained consistent pressure as much as possible. Transmission gel was applied for all scans to enhance acoustic coupling and minimize pressure on the skin. Muscle thickness was measured as the linear distance between the two aponeuroses, fascicle length was measured using the segmented line tool, and pennation angle was defined as the insertion angle between the fascicle and the proximal aponeurosis using ImageJ software (1.52v; National Institutes of Health). BF data have been already reported elsewhere (5).

Lower back pain assessment. A visual analogue pain score (VAS pain score) was assessed during the relaxed supine position. Participants were asked about subjective acute LBP they experienced in the last 30 min of bed rest by 10-point horizontal VAS (0…full absence of pain; 10…very severe pain) (31). The assessment was conducted once daily between 10:00 and 11:00 at the same time as the TMG assessment

Vertical jumping performance. For assessing vertical jumping performance, a KISTLER Quattro jump (Type 9290CD; Kistler Instrument Corp., Winterthur, Switzerland) force platform was used. The vertical jump performance was assessed after the standardized warm-up routine consisting of 6-min of stepping-up and 5 min of whole-body dynamic stretching. After the warm-up routine, 2–3 warm-up countermovement jumps (CMJ) and squat jumps (SJ) were executed, followed by actual 3 maximal jumps. Briefly, the participants stood with their hands on their hips on the force platform. In SJ, participants started from the half squat static position with their knees at approximately 90°. In CMJ, participants started from a standing position. The participants were told to jump as high as possible in each of the maximal trials. A minute break was between each maximal jump. The intra-rater reliability for CMJ and SJ jumping performance was 0.966 (95%CI, 0.901–0.991) and 0.984 (95%CI, 0.953–0.996), respectively. The highest jump was considered for further analysis. Participants were also instructed to take off and land at the same spot and to perform a triple extension (hip, knee, and ankle extension) during the flight phase, with the same full extension on landing.

Physical activity habits. A Global Physical Activity Questionnaire (GPAQ) was used to assess physical activity habits at BDC and for 6 wks during the COVID-19 lockdown period, where an average was taken for further analysis. Daily time participants spend sitting or reclining, overall weekly METs, and classification of their physical activity level (low, moderate, and high) were considered.

Statistical Analysis

Descriptive statistics have been reported as mean (standard deviation, SD). Normality of distribution was confirmed by visual inspection (histogram and Quantil-Quantil plot) and analytical tests (skewness, kurtosis, and Shapiro-Wilk test). No relevant discrepancy from normal distribution was confirmed for all the variables. Therefore, a Leven test was used to confirm the homogeneity of variance. A jumping performance reliability was checked by intraclass correlation coefficient, with 95% confidential interval. To directly compare statistical and practical significance, we have compared anthropometric and jumping variables (Table 1) as well as muscles TMG contractile parameters (Table 2), at only end-time points (BDC, BR10, R+30, and COVID-19) by repeated measures analysis of variance (RM ANOVA) with Bonferroni *post hoc* analysis. Where significant time effect was confirmed, a detailed analysis was performed by multiple RM ANOVA. If assumption of sphericity was violated a Greenhouse–Geisser correction was used. Important, as we had three opposite processes in longitudinal research design (declines after the bed rest, improvements after recovery, and again decline owing to COVID-19 lockdown), we have divided assessment time points into three groups: A bed rest effect was tested using BDC and all BRi time points; a recovery effect was tested using BDC, BR10, R+2, and R+30 time points; and COVID-19 lockdown effect was tested using BDC, BR10, R+30, and COVID-19 time points. The Pearson correlation analysis was used to correlate changes in CMJ jumping performance and TMG Tc. The level of significance was set at $P \leq 0.05$, whereas in *post hoc* analysis a P value was corrected by Bonferroni correction to $P \leq 0.017$ for bed rest effect and to $P \leq 0.017$ for recovery and COVID-19 lockdown effects. Data analysis was performed using SPSS software (version 29.0, IBM Corp., Armonk, New York). The datasets generated during and analyzed during the current study are available in the repository, [http://hdl.handle.net/20.500.12556/DiRROS-22783 (PID: 20.500.12556/DiRROS-22783)] upon reasonable requests after acceptance of the manuscript for publication.

RESULTS

All participants went through the bed rest study and assessment without complications. They all managed to adhere to the recovery plan and arrived at the assessments during the lockdown period. The assessment during the strict COVID-19 lockdown period was not initially planned; however, we managed to include that time-point through subsequent ethical approval and present the results during that period in the present manuscript.

Anthropometry and Jumping Performance

Table 1 presents participants' anthropometric data and jumping performance. At BR10, participants lost 1.5 kg owing to increased fat mass by 3.8%, whereas lean mass decreased by 0.76 kg. As all anthropometric parameters recovered at R+30 to BDC values, the fat mass increased again at COVID-19 by 3.9% compared with BDC. The observed reduction exceeded the coefficient of variation of the device (<2%), indicating that the change reflects a true physiological loss rather than measurement variability.

TABLE 1. Anthropometric characteristics, jumping performance, and physical activity habits at baseline (BDC), after 10 d of bed rest (BR10), after 30-d recovery (R+30), and during the COVID-19 lockdown.

	BDC	BR10	R+30	COVID-19	p_{Time} (η^2)
Anthropometry					
Body height/m	1.81 ± 0.039	1.82 ± 0.039	1.81 ± 0.039	1.81 ± 0.039	0.067
Body mass/kg	77.5 ± 10.0	76.0 ± 9.4*	77.1 ± 9.9	79.0 ± 10.3	0.007 (0.423)
Fat mass/%	12.6 ± 1.28	16.4 ± 1.23*	13.9 ± 1.19	16.5 ± 1.04*	<0.001 (0.824)
Muscle mass/kg	32.04 ± 2.98	31.28 ± 2.75*	32.10 ± 2.99	31.92 ± 3.06	0.005 (0.445)
Jumping performance					
CMJ height/cm	33.6 ± 4.61	30.2 ± 0.46*	32.7 ± 0.44	31.7 ± 2.88	0.004 (0.389)
CMJ P_{rel}/W/kg	49.2 ± 6.11	47.3 ± 6.59	48.9 ± 6.27	47.4 ± 5.77	0.179
SJ height/cm	31.1 ± 5.66	29.2 ± 4.86		30.2 ± 3.74	0.160
SJ P_{rel}/W/kg	51.4 ± 6.51	47.7 ± 5.89*		49.0 ± 5.93	0.018 (0.361)
Physical activity habits					
ST/min/d	531 ± 118			654 ± 168	0.004 (0.616)
MET/week	4833 ± 2067			2513 ± 2370	0.003 (0.631)
PA level of participants	90% High 10% Moderate			20% High 20% Moderate 60% Low	

Values are means ± SD; $n = 10$.

*…different from BDC at $P < 0.05$.

P_{rel}, relative jumping power; ST, sitting time; MET, metabolic equivalent; PA, physical activity.

Comparisons made with repeated measures analysis of variance (four time points) with Bonferroni *post hoc* correction.

TABLE 2. Changes in tensiomyographic Td, Tc, Dm (only in vastus lateralis), and muscle architecture from baseline (BDC) to the BR10, to R+30, and to the COVID-19 lockdown.

	BDC	BR10	R+30	COVID-19	P (η²)
Vastus lateralis					
Td/ms	22.6 ± 1.32	23.5 ± 1.40			0.006 (0.586)
Tc/ms	24.0 ± 3.50	24.5 ± 1.96			0.451
Dm/mm	6.49 ± 1.40	7.40 ± 1.17			0.019 (0.472)
MT/mm	2.60 ± 0.46	2.42 ± 0.47[a]			0.004 (0.613)
PA/deg	17.8 ± 2.23	17.0 ± 2.76[a]			0.014 (0.508)
Lf/mm	8.46 ± 0.71	8.43 ± 0.82[a]			0.819
Vastus medialis					
Td/ms	22.6 ± 0.95	22.8 ± 0.81	23.1 ± 2.73	23.5 ± 1.41	0.897
Tc/ms	24.0 ± 1.69	23.9 ± 1.18	23.5 ± 2.73	23.9 ± 1.90	0.976
Gastrocnemius medialis					
Td/ms	21.6 ± 1.39	25.2 ± 2.13	22.8 ± 1.36	25.1 ± 2.61	<0.001 (0.455)
Tc/ms	25.2 ± 5.19	34.9 ± 5.18	25.4 ± 5.84	29.8 ± 6.56	<0.001 (0.501)
MT/mm	1.79 ± 0.22	1.87 ± 0.24			0.767
PA/deg	22.5 ± 3.08	23.2 ± 4.11			0.437
Lf/mm	5.14 ± 0.53	5.01 ± 0.67			0.233
Biceps femoris					
Td/ms	24.8 ± 1.36	25.41 ± 2.56	27.0 ± 3.29	27.1 ± 3.65	0.044 (0.186)
Tc/ms	32.3 ± 6.51	38.7 ± 6.51	37.7 ± 10.5	41.9 ± 9.90	<0.001 (0.295)
MT/mm	1.98 ± 0.28	2.03 ± 0.22			0.560
Tibialis anterior					
Td/ms	23.5 ± 1.45	23.5 ± 1.60	24.8 ± 2.96	25.0 ± 2.71	0.004 (0.249)
Tc/ms	29.8 ± 14.6	28.9 ± 16.6	22.3 ± 9.36	27.8 ± 13.7	0.243
MT/mm	2.66 ± 0.30	2.61 ± 0.30			0.109
PA/deg	9.33 ± 1.43	9.25 ± 1.33			0.676
Lf/ mm	8.10 ± 1.80	8.03 ± 1.37			0.812
Erector spinae					
Td/ms	21.0 ± 2.33	18.0 ± 2.03	22.0 ± 2.45	20.8 ± 3.00	<0.001 (0.505)
Tc/ms	16.7 ± 1.98	14.1 ± 1.40	16.0 ± 0.97	14.8 ± 1.54	<0.001 (0.539)

Values are means ± SD; n = 10.
[a]Owing to muscle biopsy, an assessment was taken at BR9.
Lf, fascicle length.
Comparisons made with repeated measures analysis of variance (two or four time points) with Bonferroni *post hoc* correction.

At BR10, when compared with BDC, CMJ height decreased by 10.1% and returned to BDC values at R+30, whereas CMJ relative power remained unchanged. No changes were observed in SJ height at BR10, whereas SJ relative power decreased by 7.2% at BR10 and returned to BDC values at R+30. There was no change in both jumping performances during COVID-19.

Physical activity habits changed during the COVID-19 period. Sitting time increased by 23.2%, and weekly metabolic equivalent, a measure of energy consumption owing to physical activity and exercise decreased by 48%, resulting in 60% of participants in "low" physical activity level.

Tensiomyographic Delay and Contraction Time

Table 2 presents statistical analysis of TMG-derived Td and Tc in all muscles at four main time points (BDC, BR10, R+30, and COVID-19). Where the main time effect was confirmed, a detailed analysis was performed (Figure 1). In VM Td and Tc, no changes were detected during the entire period. TA showed similar behavior, as there was no significance found in *post hoc* analysis. In contrast, VL Td increased at BR10 for 4.0% (P = 0.006), whereas Tc remained unchanged. In the other muscles, a detailed analysis was performed and presented in Figure 1 with appropriate *post hoc* P values.

Taking into account all time data points (Figure 1), Td does not change during the bed rest period in BFlh, while it increases in GM at BR4 (11.2%; P = 0.008), peaks at BR10 (16.7%; P = 0.002), remains elevated at R+2 (9.3%; P = 0.002), and returns to BDC at R+30. Interestingly, GM Td increases again during the COVID-19 lockdown period (16.6%; P < 0.001). Opposite to the increase in VL and GM, ES Td decreases already at BR3h (2.9%; P = 0.004) peaks at BR10 (14.6%; P < 0.001), and recovers to BDC already at R+2 and remains the same also in R+30 and COVID-19.

In GM and BFlh, Tc increases from BR3h (15.7%; P = 0.001) and BR4 (14.3%; P = 0.006), respectively. GM Tc peaked at BR10 (38.4%; P < 0.001), remained increased at R+2 (22.9%; P = 0.004), and then decreased back to BDC. The increase during the COVID-19 lockdown is close to significance (P = 0.029). However, in BFlh Tc, the increase remains throughout all three periods, at BR10 (19.9%; P = 0.009), at R+30 (19.9%; P = 0.009), and at COVID-19 (29.9%; P = 0.002). In ES, Tc decreased at BR2 (16.0%; P = 0.002), peaking at BR6 and BR8 (17.5%; P = 0.001), recovered already at R+2 and decreased again at COVID-19 (12.1%; P = 0.007).

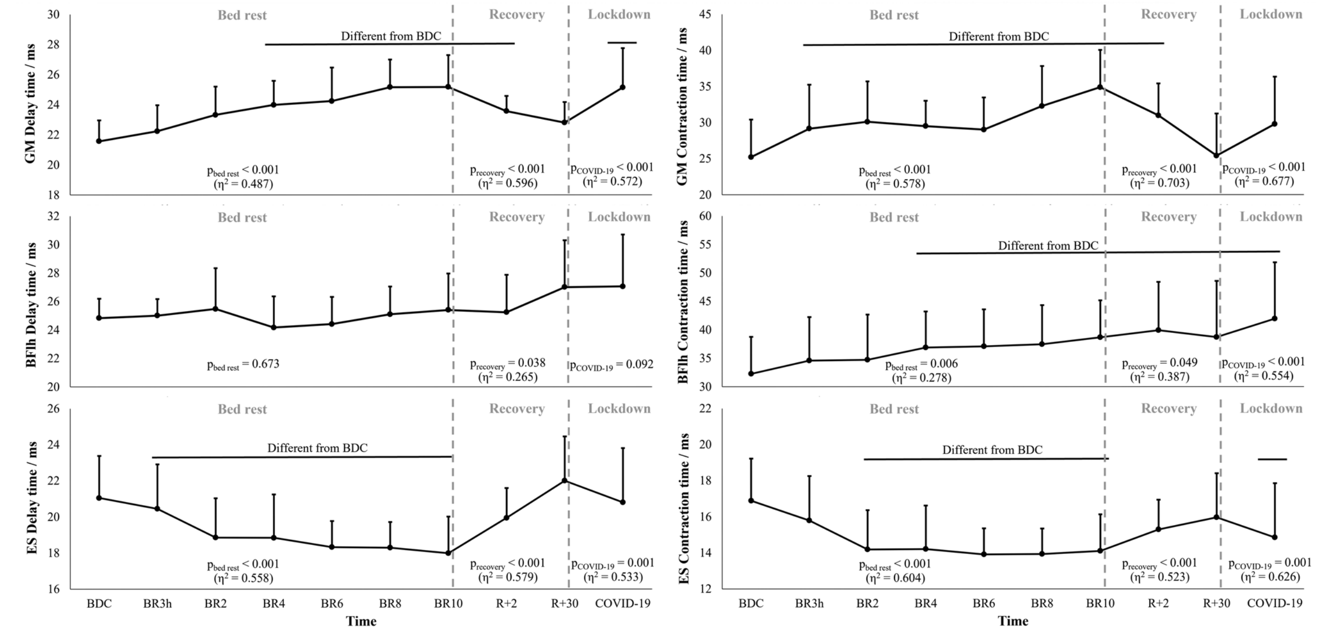

FIGURE 1—Changes in tensiomyographic delay and contraction time during the bed rest, recovery, and lockdown periods. Data are presented for three muscles: GM, BFlh, and ES. Values are means ± SD; *n* = 10; $p_{bed\ rest}$ …significant time effect during the entire bed rest period (BDC—baseline; BR3h—3 h after BDC; BRi—i-days after BDC); $p_{recovery}$… significant time effect during the recovery period (BDC; BR10, R+2—2 d after the bed rest; R+30—30 d after the bed rest); $p_{covid-19}$… significant time effect during the lockdown period (BDC; BR10, COVID-19—during the COVID-19 lockdown). Straight line indicates *post hoc* differences between overlined time points and the BDC. Comparisons made with repeated measures analysis of variance, separately for bed rest, recovery, and COVID-19 lockdown effect, each with Bonferroni *post hoc* correction.

Tensiomyographic Amplitude

In all five leg muscles (VL, VM, BFlh, GM, and TA) Dm increased during the bed rest period, decreased toward BDC levels during the recovery period, and increased again during the COVID-19 lockdown period but to a lesser extent (Figure 2). However, in ES Dm decreased at the initial phase of the bed rest period (BR3h and BR2) and later remained unchanged compared with BDC. Specifically, VL Dm increased by 14.1% ($P = 0.019$) at BR10 when compared with the BDC (Table 2), as we did not assess at other time points, as described in the methods. However, VM Dm increased already after 3 h of bed rest, at BR3h (9.1%; $P = 0.008$), highest at BR10 (27.6%; $P < 0.001$), decreased to the BDC already at R+2 and increased again at COVID-19 (10.3%; $P < 0.001$). BFlh Dm increased also already at BR3h (18.6%; $P = 0.003$), highest at BR10 (41.5%; $P < 0.001$), decreased to the BDC at R+30 and increased again at COVID-19 (19.9%; $P = 0.014$). GM Dm increased at BR4 (46.6%; $P = 0.003$), highest at BR10 (64.4%; $P < 0.001$), decreased to the BDC at R+2, and increased at COVID-19 (24.9%; $P = 0.025$). TA Dm increased at BR3h (16.4%; $P = 0.006$), highest at BR10 (46.8%; $P < 0.001$), decreased to the BDC at R+2, and did not increase at COVID-19. In contrast, the ES Dm decreased at BR3h (12.1%; $P < 0.001$), more at BR2 (27.4%; $P < 0.001$), increased back to the BDC at BR4, and remained unchanged throughout the bed rest and recovery periods and tended to decrease again at COVID-19 (18.1%; $P = 0.054$).

Lower Back Pain Perception

VAS scale confirmed lower back pain elevation at BR1 and BR2 in all participants when compared with BDC (Figure 3). Later, the pain disappeared, and was not present after BR4, either in recovery or lockdown periods. The increase in lower back pain was paralleled by a decrease in ES Dm, which is the opposite of what we observed in leg muscles during bed rest. No correlations were found between the increase in lower back pain and any TMG parameters (ES Dm decrease, ES Td decrease, or ES Tc decrease), either at BR3h or at BR2.

Muscle Architecture

VL thickness decreased at BR9 (7.0%; $P = 0.004$; Table 2 and Figure 4) and remained decreased also at COVID-19 (10.1%; $P = 0.009$). In parallel, VL pennation angle decreased also at BR9 (4.5%; $P = 0.014$; Table 2), while fascicle length remained unchanged. In all other muscles (GM, BF, and TA), muscle architecture did not change during the bed rest period.

Correlation between Changes in Jumping Performance and TMG Contraction Time

A negative correlation was found between GM Tc and CMJ jumping height (r = -0.672; 95% CI, -0.915 to -0.173, $P = 0.033$; Figure 5).

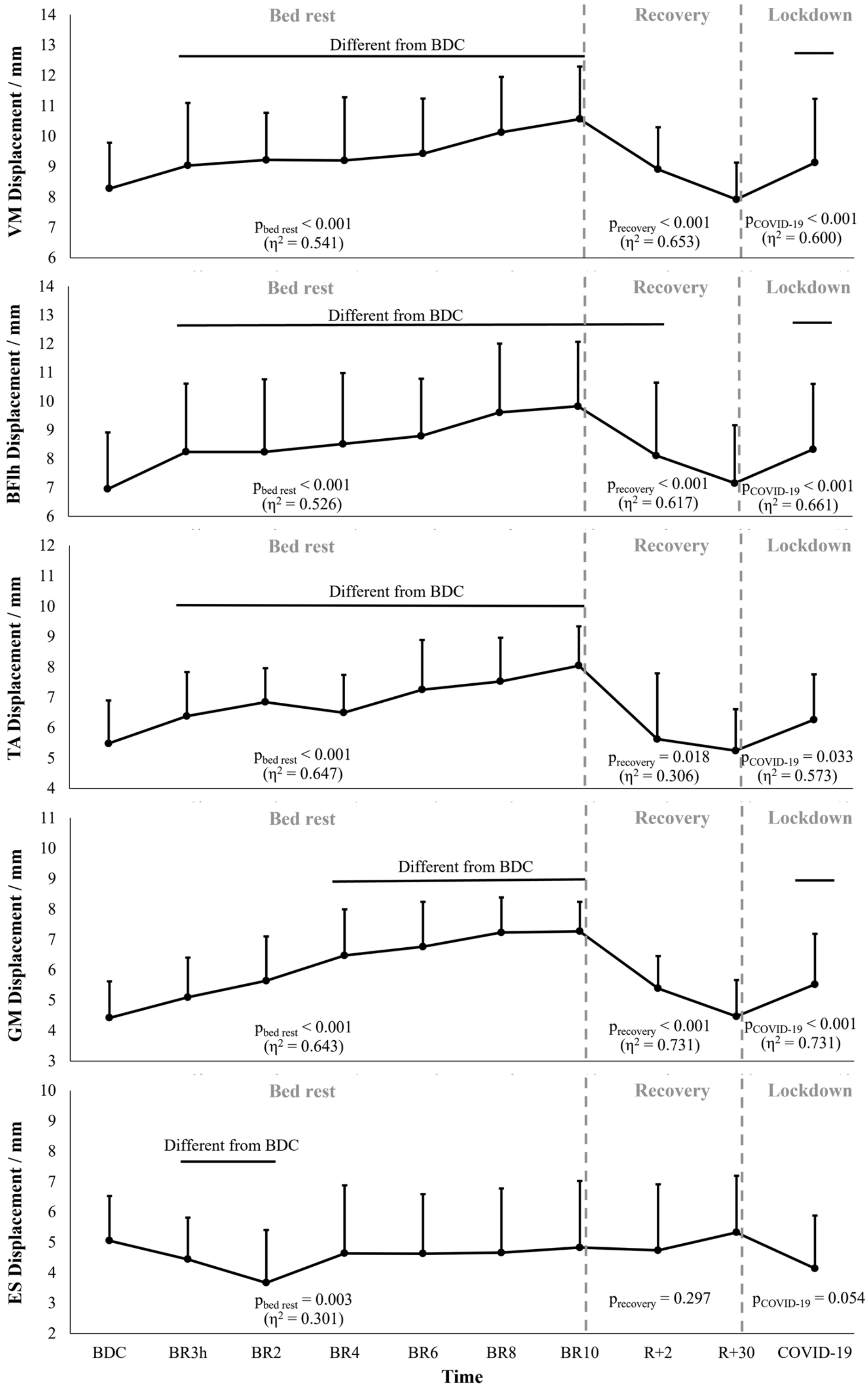

FIGURE 2—Changes in tensiomyographic amplitude during bed rest, recovery, and lockdown periods. Data are presented for all five muscles: VM, TA, GM, BFlh, and ES, assessed in all time points. Values are means ± SD; *n* = 10; $p_{bed\ rest}$ …significant time effect during the entire bed rest period (BDC—baseline; BR3h—3 h after BDC; BRi—i-days after BDC); $p_{recovery}$… significant time effect during the recovery period (BDC; BR10, R+2—2 d after the bed rest; R+30—30 d after the bed rest); $p_{covid\text{-}19}$… significant time effect during the lockdown period (BDC; BR10, COVID-19—during the COVID-19 lockdown). Straight line indicates *post hoc* differences between overlined time points and the BDC. Comparisons made with repeated measures analysis of variance, separately for bed rest, recovery, and COVID-19 lockdown effect, each with Bonferroni *post hoc* correction.

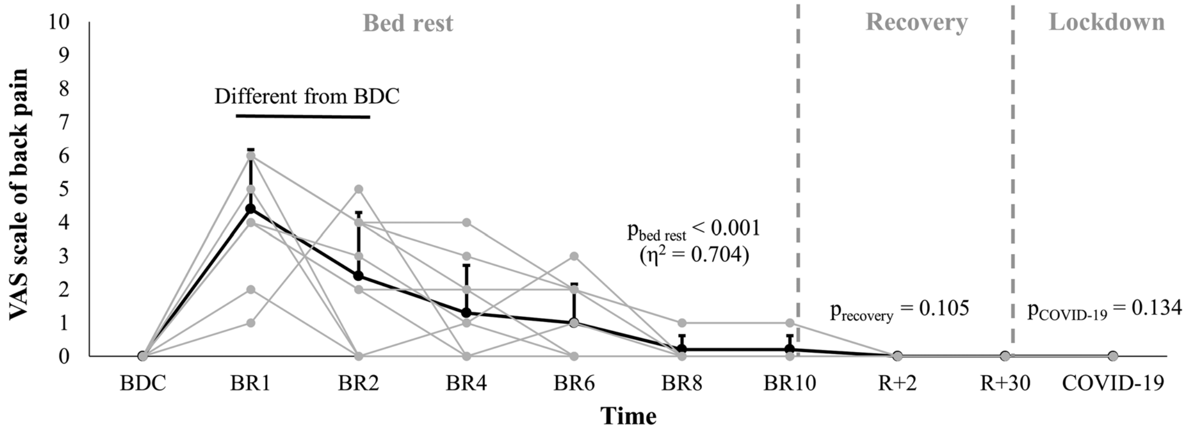

FIGURE 3—Changes in VAS for lower back pain during bed rest, recovery, and lockdown periods. Values are means ± SD; *n* = 10; $p_{bed\ rest}$ … significant time effect during the entire bed rest period (BDC—baseline; BR3h—3 h after BDC; BRi—i-days after BDC); $p_{recovery}$… significant time effect during the recovery period (BDC; BR10, R+2—2 d after the bed rest; R+30—30 d after the bed rest); $p_{covid\text{-}19}$… significant time effect during the lockdown period (BDC; BR10, COVID-19—during the COVID-19 lockdown). Straight line indicates *post hoc* differences between overlined time points and the BDC. Comparisons made with repeated measures analysis of variance, separately for bed rest, recovery, and COVID-19 lockdown effect, each with Bonferroni post hoc correction.

DISCUSSION

Effect of Bed Rest

In our study, we showed that 10 d of horizontal bed rest led to a lean mass loss of 0.76 kg and decreased CMJ height by 10.1%, without changes in CMJ relative power. We have previously reported changes in other muscle mass and force-related metrics at BR10, compared with BDC, in particular, we observed a decrease of 5.2%, 14.3%, and 10.3% in quadriceps muscle volume, maximal voluntary contraction, and muscle tension (maximal voluntary contraction/CSA), respectively (23). In addition to this previous study, here we also provide evidence that total lean mass and jumping performance returned to baseline after 30 d of recovery.

Although ultrasound revealed changes in muscle architecture occurring from BR6 for BFlh (5) and from BR9 for VL, the TMG amplitude (Dm) of BFlh increased already at BR3h (18.6%) and peaked at BR10 (41.5%). In VL, an increase at BR10 was also confirmed; however, we did not assess further time points. Very similar patterns were found in VM and TA, while in GM Dm increased at BR4. Conversely to lower limb muscles, ES Dm decreased at BR3h and BR2 compared with BDC. In the other muscles, architectural parameters remained unchanged after 10 d of bed rest, likely because a longer unloading

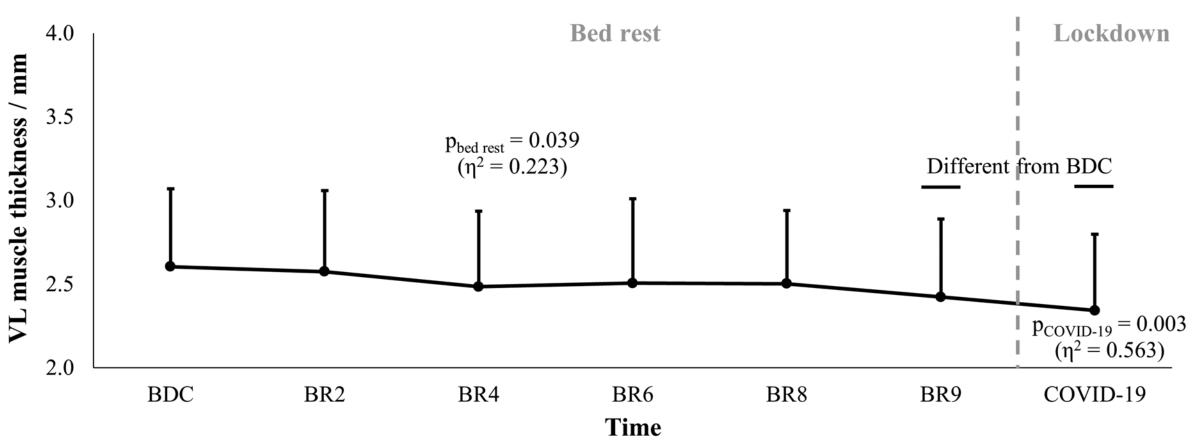

FIGURE 4—Changes in VL thickness during the bed rest, recovery, and lockdown periods. Values are means ± SD; *n* = 10; $p_{bed\ rest}$ …significant time effect during the entire bed rest period (BDC—baseline; BR3h—3 h after BDC; BRi—i-days after BDC); $p_{covid\text{-}19}$… significant time effect during the lockdown period (BDC; BR10, COVID-19—during the COVID-19 lockdown). Straight line indicates *post hoc* differences between overlined time points and the BDC. Comparisons made with repeated measures analysis of variance, separately for bed rest effect, and COVID-19 lockdown effect, each with Bonferroni *post hoc* correction.

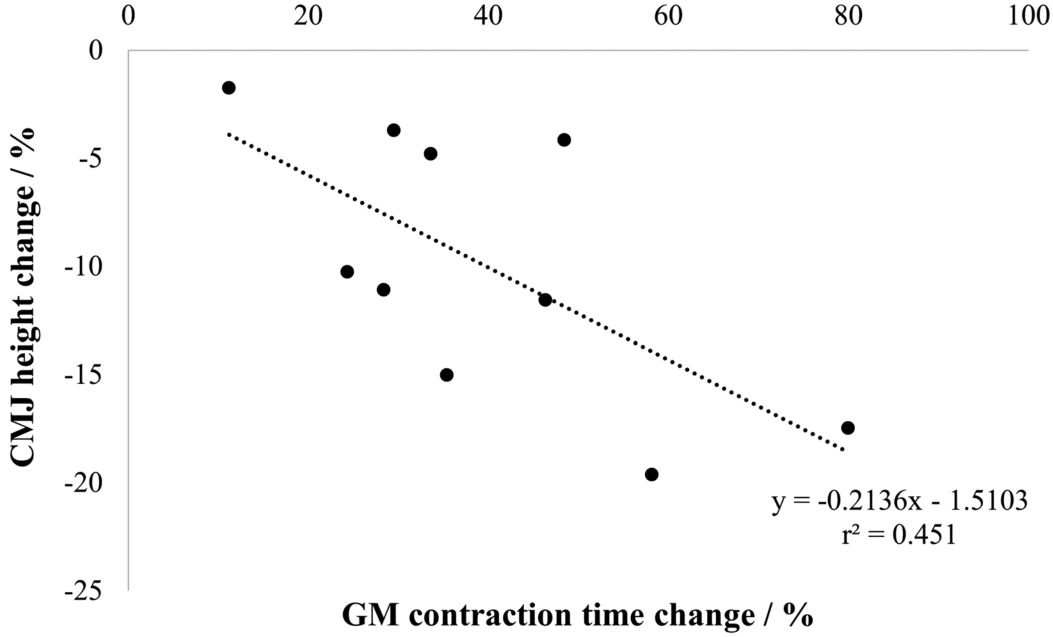

FIGURE 5—A correlation with linear regression analysis of bed rest increments of GM contraction time and decrements of countermovement jumping height. *N* = 10; straight dotted line indicates a linear trend (*y*) between both variables. A determination coefficient (r^2) estimates a common variance of both variables based on Pearson correlation coefficient.

duration is required to exceed the inter-day minimal detectable change for muscle thickness, which ranges from 0.9 mm in VL to 1.2 mm in GM for an experienced examiner (32). Similar to previous studies, we observed an increase in Dm within 35 d of bed rest in all the muscles, though the increase in HDT was more pronounced (7) than in horizontal bed rest (20). Early and substantial increase in leg muscle's Dm could be explained by well-known fluid shift, from the legs toward the head (33), as small changes in muscle fluid volume significantly alter passive muscle tension (34). Further, it is also known that 24 h of dry immersion induce a decrease in muscle stiffness and muscle tone, which increases transversal muscle oscillations during voluntary and electrically evoked contractions (35). Already within the first few minutes of HDT bed rest, there is a shift of fluid from the extremities to the chest when the leg volume decreases from 7.5 to 6.9 l (36). This could be confirmed by the observation that Dm was already elevated in BFlh after as little as 3 h of bed rest, before any overt architectural changes and muscle atrophy had taken place (5).

In contrast to the changes observed in the lower limb muscles, ES Dm was reduced within the first 2 d of bed rest and this was paralleled by increased LBP. LBP during bed rest is induced by reduced amplitudes of spine movements rather than by increases in the spine length. It is suggested that isometric muscle contractions of low intensity are a primary course of back pain (37). An increase in LBP was found to be associated with greater resting ES muscle stiffness, assessed by shear-wave elastography (38). Our results suggest that LBP increases muscle resting stiffness, possibly owing to increased tonic activity during muscle spasms. To support this, decreased ES Td and Tc are confirming muscle preactivation, most likely pre-activation of type I muscle fibers. The sensitivity of TMG to detect pain-related adaptations of lower back muscles is of high importance in clinical and microgravity settings. LBP often precedes structural or functional changes in spinal stabilizing muscles (e.g., multifidus, ES) and early detection allows timely intervention before chronic changes occur. LBP is one of the most common complaints in astronauts, where 70% of astronauts report LBP during missions or immediately upon return (21). However, LBP is self-limiting in spaceflight and should not pose an operational risk. Changes in TMG- or twitch dynamometry-derived Td or latency time after bed rest periods were not previously reported. Our findings are not consistent throughout the measured muscles, as we have found increased Td in VL and GM, no change in VM, BFlh, and TA, and a decrease in ES. Although decreased ES Td could be owing to muscle spasm and muscle elongation, there is no clear mechanism to explain inconsistencies of Td change in other muscles. The latency time reflects a neuromuscular delay from the stimulus delivered, action potential generation, and time used by the action potential to reach the muscle until the muscle develops tension. This time lag reflects the time required to accomplish several biochemical and mechanical tasks, including calcium diffusion out of the sarcoplasmic reticulum and binding to troponin, the movement of tropomyosin off the active sites, formation of cross bridges, and taking up any slack that may be in the muscle, where initial slack depends on changes in muscle and tendon stiffness, also affected by bed rest (39). Lower Td in ES, starting from the LBP period of early bed rest, confirms such hypothesis. Important here, when Td is calculated from TMG is 23.4% shorter than when calculated from torque twitch response, owing to a more sensitive measurement approach in TMG as the mechanical response is detected at a muscle belly directly and not distally as a torque (40).

We have found increased Tc in GM and BFlh, decreased Tc in ES, and unchanged Tc in the remaining muscles (VM, VL, TA). Our team previously reported unchanged Tc in VM and increased Tc in BFlh after 35-d HDT bed rest without recovering at R+30 (7). We have reported even earlier BFlh Tc increase in this study owing to higher statistical power (less consecutive assessments) (5). A novel finding from the present work is also immediate but reversible increase in GM Tc.

Previously we have found a correlation between Tc decrease with improvements in jumping performance after 8-wk plyometric training (41). The functional importance of Tc for explosive movements was also confirmed in this study, as we have found a negative correlation ($r = -0.672$; $P = 0.033$) between increments in GM Tc and decrements in CMJ height after bed rest. However, even with confirmed normality and no identified outliers, caution is warranted because the small sample size results in wide 95% confidence intervals for the correlation coefficient.

Effect of Recovery from Bed Rest

All anthropometric, jumping, and TMG parameters returned to baseline at R+30, except BFlh Tc. Since 30 d of recovery, after 10-d bed rest, is a long and effective rehabilitation time frame for younger participants, the full recovery of all main neuromuscular parameters were expected (14). Increased BFlh Tc was also confirmed after 35-d HDT bed rest, without recovery to BDC after 30-d recovery in young males (7). Although, it is well known that quadriceps atrophy more than hamstrings during the bed rest (42), it is less known about intermuscular variability during the recovery after bed rests. However, adaptations after bed rest extend beyond muscle atrophy. Neural adaptations—including reduced spinal excitability, altered reflexes, and impaired integration of proprioceptive, visual, and vestibular inputs—contribute to decreased stability during early reambulation (43). Consequently, functional recovery of muscles, such as hamstrings, may be delayed by persistent neuromotor deficits that limit activation and recruitment during daily activities. For example, lower BFlh Tc correlates with higher vertical jump in young (41) and old (44) participants, advocating its role in physical exercise and performance. The increase in BFlh Tc, which we found after bed rest, is also found in aging muscle, already after 35 yr of age in the general population as well as in master athletes, to a lesser extent in explosive athletes and to a higher extent in endurance athletes (45). This may have significant clinical implications for the quality of life after hospitalization. Identification of atrophic muscles may therefore also provide important information for the design and optimization of personalized exercise interventions to target specific muscle groups (46).

Effect of COVID-19 Lockdown

We observed physical activity changes comparable to those reported in the multicentric study (22), even though our study was originally designed to examine the effects of bed rest, and the comparison with COVID-19 lockdown data is only exploratory. COVID-19 changed the lifestyle by Slovenian population for long periods, by increasing periods of sitting time by 28.6%, diminishing overall physical activity by 38%, diminishing walking time by 42.7%, diminishing moderate and vigorous physical activity by 34.7% and 36.9%, respectively (22). Similar trends were found also in our sample of participants, with 23.2% increased sitting time and 48% decreased energy consumption owing to physical activity and exercise, according to physical activity questionnaires. In 2-month lockdown period our participants increased fat mass. This finding aligns with studies using milder inactivity paradigms, such as step reduction, which have shown differences in body composition and metabolic health during short periods of reduced ambulatory activity (47). In addition, TMG-derived contractile parameters changed in the same direction as at BR10; however, to a smaller extent, suggesting the sensitivity of TMG to assess more subtle changes in muscle properties. After the COVID-19 lockdown Dm of VM, GM, and BFlh increased again, after it fully recovered at R+30. Interestingly, BFlh Tc never recovered from bed rest, as it remained increased from BDC, at BR10, throughout the recovery period and after the COVID-19 lockdown by 30%. This makes TMG suitable for studying muscle contractile properties in more subtle disuse models or during realistic periods of disuse.

Study Limitations

The study was conducted on a small sample consisting exclusively of male participants, which is a common limitation in bed rest research. To ensure a homogeneous sample and optimize statistical power within the group of ten participants, inactive individuals and elite athletes were excluded. This approach supported the main study objectives reported in other project publications but may limit the generalizability of the findings to recreationally active males. The exploratory COVID-19 lockdown assessments were not pre-planned and therefore not included in the power analysis. We have presented changes in body composition by less accurate bioimpedance, as we did not measure MRI (for muscle mass) after COVID-19 lockdown. However, we have presented muscle volume changes after the bed rest being published elsewhere (23). In a VL muscle, we could not follow the same repeated assessment owing to a muscle biopsy being taken from the very same muscle before and after the bed rest study, with additional biopsy on a contralateral leg at mid bed rest. Physical activity during the COVID-19 lockdown was assessed via telephone interview using the GPAQ, as travel across the country to provide participants with more accurate accelerometry devices was not permitted.

CONCLUSIONS

The results of this study provide evidence that elevated tensiomyographic amplitude (Dm) enables early detection of muscle atrophy before morphological alterations. An exception was observed in the ES muscle, where LBP was associated with reduced Dm. Furthermore, tensiomyographic Tc in the BFlh increased during bed rest and remained persistently elevated throughout recovery and the COVID-19 lockdown. These findings suggest the same trend in TMG changes during bed rest and after COVID-19 lockdown, albeit with smaller magnitude. These findings support the utility of TMG as a sensitive and noninvasive method for monitoring muscle responses across different disuse paradigms. Given the noninvasive nature of this technique and its low-cost, its use seems of relevance in clinical settings where an early detection of changes in muscle mass and function are needed to improve diagnosis and treatment of bed-ridden patients.

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