TITLE:

Effect of 10 days of unilateral lower limb suspension on knee extensors neuromuscular function and spinal excitability

RUNNING TITLE:

Muscle disuse and neuromuscular function

AUTHORS:

Loïc Lebesque[1], Marco V. Narici[2,3], Alain Martin[1], Giuseppe De Vito[2,3], Fabio Sarto[2] and Gil Scaglioni[1]

AFFILIATIONS

[1] INSERM UMR1093-CAPS, Université Bourgogne Franche-Comté, UFR des Sciences du Sport, F-21000, Dijon.

[2] Department of Biomedical Sciences, University of Padova, Padova, 35131, Italy.

[3] CIR-MYO Myology Center, University of Padua, 35131, Italy.

CORRESPONDING AUTHOR: Address for reprint requests and other correspondence:

Gil Scaglioni

INSERM UMR1093 "Cognition, Action et Plasticité Sensorimotrice »

Université de Bourgogne - UFR STAPS

Campus Universitaire, BP 27887 DIJON

Phone: +33 3 80 39 67 58

E-mail : gil.scaglioni@u-bourgogne.fr

ABSTRACT

The reduction in mechanical loading applied on the lower limb has numerous detrimental consequences on neuromuscular function. The current study aimed to investigate the changes in knee extensors' strength and spinal excitability induced by unilateral lower limb suspension (ULLS), providing new insights into the neuromuscular adaptations to muscle hypoactivity. Ten young healthy males (19-28 years old) underwent 10 days of ULLS to simulate muscle disuse. Modulation by unloading of knee extensors' function (muscle morphology and strength, activation capacity and contractile properties) and spinal reflexes were explored before and after the ULLS. The knee extensors' anatomical cross-sectional area (-4%, $p = 0.007$), maximal strength (-27%, $p < 0.001$) and central activation ratio (-3%, $p = 0.006$) were reduced after 10 days of ULLS. Vastus medialis H-reflex amplitude was enhanced both at rest (+33%, $p = 0.038$) and during a low-intensity contraction set at 10% of maximal strength (+103%, $p = 0.038$). No changes in muscle contractility and nerve conduction velocity were observed after the ULLS. The present study suggests that neural impairments mainly contribute to the decrease in knee extensors' strength induced by short-term ULLS. The decrease in muscle activation after a short period of ULLS was accompanied by an increase in spinal excitability. However, the latter adaptation did not counterbalance the reduction in activation capacity and thus in maximal strength resulting from ULLS. These adaptations to short-term ULLS should be considered when aiming at improving the neuromuscular function of people experiencing muscle hypoactivity.

NEW & NOTEWORTHY (75 words)

This study provides new insights into the effects of muscle hypoactivity on neuromuscular function and spinal excitability in the major antigravity muscle group of the lower limb. Neural impairments primarily contribute to maximal strength loss after short-term unilateral lower

limb suspension, while spinal excitability increased. These findings are crucial as they offer valuable understanding for developing effective interventions to improve health outcomes for individuals experiencing muscle inactivity.

KEY WORDS

Atrophy; Disuse; Force control; H-reflex; T-reflex; Unloading; Vastus medialis

75 INTRODUCTION

76 Reductions in mechanical loading may occur as a consequence of surgery, injury and
77 illness (1–3), but also as a result of reduced daily physical activity (4,5) or gravitational input, as
78 during spaceflight (6). The direct outcome is a loss of muscle mass and function, associated
79 with changes in motor control (7,8).

80 Several models of immobilization allow to explore the neuromuscular impairment in
81 response to disuse (8). However, unilateral lower limb suspension (ULLS) enables to restrain
82 atrophy to the explored musculoskeletal segment, easily mimicking the joint unloading that
83 follows injuries or surgery (7,9) and, compared to casting, has the advantage of being less
84 binding.

85 Extensive literature shows that the extent of muscle weakness is greater than the
86 decrease in muscle mass since the first few days of ULLS, meaning that neuromuscular function
87 is rapidly impaired by disuse (7,9–12). Among the antigravity muscles, the knee extensors
88 appear to be the most affected by ULLS (13–15), showing a strength loss considerably larger
89 than that of the plantar flexors (-1.0% · day^{-1} *vs.* 0,7% · day^{-1}, for durations between 10 and 35
90 days of ULLS; 8,11). Therefore, although it is reasonable to assume that for unloading, as for
91 overloading, changes in neural drive are the first to occur (8), their involvement in the
92 weakening of antigravity muscles, associated with disuse, is still an unresolved issue. Although
93 studies have shown that ULLS (21-30 days) does not interfere with the central motor drive of
94 knee extensors muscles (9,11,15,16), others have found a decrease (17,18) or even an increase
95 (range + 0.1%.day^{-1} increase to − 0.2%.day^{-1} decrease; median − 0.2%.day^{-1}) of muscle
96 voluntary activation (8). This divergence was also observed in surface electromyographic (EMG)
97 data, recorded during maximal voluntary contractions, with studies showing a reduction of the
98 vastii activity after ULLS (7-42 days) (19–23), and others finding no change for the vastii and RF

(ULLS 7-35 days) (11,22,24). On the other hand, the literature agrees on the EMG data recorded during submaximal contractions. Interestingly, in this case, all studies showed that vastii EMG activity increased during submaximal contractions following ULLS (7,23–25), suggesting that additional motor units are necessary to produce the same absolute force output after unloading. In light of these considerations, it appears that changes at neural level have not yet been fully understood, and neither have the underlying mechanisms. Previous studies, on plantar flexors, have shown an unequivocal enhancement in the amplitude of the Hoffman (H)-reflex in response to unloading (7,8,12–14,26–30). The H-reflex is the EMG response evoked by electrically stimulating the Ia fibers of a peripheral nerve and reflects the efficacy of the synaptic transmission between Ia afferents and the motor neurons (31). Since it is generally considered as a comprehensive index of spinal excitability (e.g. 32,33), for greater readability we will use the term 'spinal excitability' to refer to the efficiency of the synaptic transmission between Ia afferents and motor neurons. The enhancement of H-reflex amplitude with unloading may be ascribed to an increased synaptic transmission efficiency and/or to a greater motoneuronal excitability (13,30,34), however, the improved synaptic transmission efficiency seems to be the predominant adaptation in all forms of disuse (13). This mechanism may interfere with the reduction in motoneuronal excitability that might occur together with an increased proportion of fast fibers in response to unloading (27). These adjustments should equally affect H and T-reflexes. The T-reflex is an electrophysiological variant of the stretch reflex caused by a mechanical tap applied on the tendon that, generating a quick stretch of the tendon itself, triggers muscle spindles discharge to Ia afferents notably. Both reflexes originate partly from the monosynaptic projections of the Ia afferents to alpha-motor neurones, but the T-reflex involves all the elements of the reflex pathway, including muscle spindles sensitivity and II afferents (35,36). The literature shows that the T-reflex may be further modulated by

123 conflicting changes arising in peripheral components of the stretch reflex pathway. Actually,
124 Anderson *et al.* (27) found, after 3 weeks of hind limb suspension in rat, a decline of the maximal
125 soleus T-reflex but a rise in the maximal H-reflex (H_{MAX}). Seynnes *et al.* (29) observed no change
126 in soleus T-reflex, following 23 days of ULLS, but an increase in H-reflex and a decrease in
127 tendon stiffness. The different modulations of the T-reflex response with unloading may be
128 ascribed i) to a lower spindles' sensitivity, due to their reduced solicitation during
129 immobilization (37), and/or ii) to a decreased stiffness of the musculo-tendinous elements in
130 series with the muscle spindles (27,29).

131 In view of these considerations and since the literature seems to show that few days of
132 immobilization induce significant changes in neuromuscular function (8), this study sought to
133 investigate the role of changes in spinal reflex excitability, muscle contractile characteristics
134 and muscle morphology in the loss of knee extensors' strength following 10 days of muscle
135 unloading. Since, in plantar flexors, H-reflex is always modulated by ULLS and considering that
136 knee extensors are among the antigravity muscles most affected by inactivity, we hypothesized
137 that spinal excitability would be increased and muscle mass reduced by unloading. However,
138 given the short period of immobilization, it could also be assumed that neural adaptations
139 might be preponderant over muscular changes. Therefore, apart from improving knowledge on
140 mechanisms responsible for the decline of the neuromuscular function due to unloading, the
141 outcomes of the present investigation may be useful for refining the rehabilitation strategies
142 of patients following periods of muscle disuse.

143

144

145

146

MATERIALS & METHODS

Ethical approval.

The present study was approved by the Ethics Committee of the Department of Biomedical Sciences of the University of Padova (Italy) (reference number HEC-DSB/01-18) and conducted in accordance with the standards set by the latest revision of the Declaration of Helsinki. Participants were informed about all experimental procedures through an interview and an information sheet, and could ask questions to the selection committee. After signing the written consent form, participants were enrolled in the study with the possibility to withdraw at any stage of the experiment. This study was part of a larger investigation aiming to study the neuromuscular alterations with short-term disuse (18,38).

Participants

Ten healthy and recreationally active young male volunteers (age: 22.9 ± 4.0 years old; body mass: 72.2 ± 7.2 kg; height: 177.6 ± 3.1 cm; body mass index: 22.9 ± 2.1 $kg \cdot m^{-2}$) took part to this study. Because unilateral lower limb suspension (ULLS) is associated with an increased risk of deep venous thrombosis (39), more commonly experienced in young females (40), only males were recruted in this study. Inclusion criteria were: age comprised between 18 and 35 years, body mass index comprised between 20 and 28 $kg \cdot m^{-2}$ and involvement in recreational physical activities 1-3 times per week. Exclusion criteria were: sedentary lifestyle, professional sport practice, smoking, history of deep venous thrombosis and acute or chronic musculoskeletal, metabolic and cardiovascular disorders. Using the Global Physical Activity Questionnaire (41), participants demonstrated a high level of physical activity (3268 ± 1468 $MET \cdot min \cdot week^{-1}$) in the period leading up to the ULLS experiment. Volunteers were asked to

171 maintain their habitual diet throughout the intervention period and refrain from intense
172 exercise, coffee and alcohol intake during the 24 hours preceding the data collection at each
173 time point.
174
175 Protocol
176 *Experimental design.* Data were collected the day before (LS0) and after 10 days (LS10) of
177 unilateral lower limb suspension (ULLS). For each subject, sessions lasted 2 hours and were
178 planned at the same time of the day to avoid any circadian disturbance (42,43). At both LS0
179 and LS10, the experimental procedure was performed beginning with the evaluation of the
180 maximal voluntary isometric contraction (MVC) of knee extensors and the maximal voluntary
181 activation level (VAL). Then, vastus medialis (VM) spinal excitability were assessed through
182 electrical stimulations and patella tendon striking at rest and during a weak contraction of knee
183 extensors (10% MVC assessed for each session). In addition, quadriceps and VM morphology
184 was assessed through ultrasonography, on the same day of the ULLS beginning (baseline data
185 collection, BDC) and the day before the end of the ULLS (LS9).
186
187 *Maximal force production capacity.* To start the maximal force production evaluation, subjects
188 performed a knee extensors warm-up composed of 10 submaximal contractions, lasting 4 s
189 each. Then, three knee extensors MVCs (lasting 4 s, with 1-min rest in-between) were
190 performed with maximal electrical doublet (100 Hz) delivered over the peak force.
191
192 *Spinal excitability*. Since it is more challenging to evoke rest H-reflex in the quadriceps muscles
193 compared to the soleus muscle, several studies suggest eliciting it during a weak muscle
194 contraction (44–47). The reflex response is indeed enhanced by the muscle contraction

195 because motoneurons are brought closer to their firing threshold by the voluntary drive (48).
196 In addition, muscle activation allows to keep constant the "background" level of α-motoneuron
197 depolarisation and limits post-synaptic modulations (45,49). Therefore, the assessment of
198 spinal excitability during muscle contraction has the advantage of increasing amplitude and
199 reducing variability in both latency and amplitude of the H-reflex (48,50,51). Consequently, VM
200 H-reflex and M-wave recruitment curves were built by applying electrical stimulations first
201 during weak knee extensors contractions (10% MVC) and then at rest. To build the recruitment
202 curves, stimulations started at 5 mA and were progressively increased with a 5-mA increment
203 until the maximal M-wave (M_{MAX}) was reached. To ensure that the M-wave lied in the plateau
204 of its maximal value throughout the experiment, the maximal stimulation intensity was
205 increased by 20%. Subsequently, additional stimulations were applied, in 2 mA increments,
206 around the intensity that allowed to record the highest H-reflex, in order to accurately estimate
207 the intensity needed to elicit the maximal H reflex (H_{MAX}). Four stimuli were delivered at each
208 intensity interspaced by 10 s intervals. Then, 6 out of 10 subjects underwent complementary
209 evaluation of spinal excitability at rest through the assessment of tendinous reflex (i.e., T-reflex,
210 for the method see below mechanical stimulation).
211
212 *Unilateral lower limb suspension*. The ULLS model was employed for 10 days. The non-dominant
213 leg (left leg for all participants) of the participants was fitted with a shoe having an elevated
214 sole (50 mm), while the dominant leg (right leg for all participants) was suspended and kept at
215 a slightly flexed position (approximately 15-20° of knee flexion) using straps. Volunteers were
216 asked to walk on crutches during the whole ULLS period and to refrain from loading the
217 suspended leg in any way. A familiarization session was set up for the participants to practice
218 daily tasks with the limb suspended (9). Participants were recommended to wear elastic

219 compression socks on the suspended leg during ULLS period and to perform passive, range of
220 motion, non-weight-bearing exercises of the ankle, as precautionary measures to prevent deep
221 venous thrombosis (39). Moreover, we performed an ultrasound-Doppler examination after 5
222 days of ULLS. Participants' compliance was evaluated through daily calls and messages and by
223 comparing calves' temperature and circumference after 5 and 10 days of ULLS, as previously
224 suggested (9).

225

226 Data acquisition.

227 *Ultrasonography.* The morphology of the quadriceps was assessed by ultrasonography, which
228 also allowed us to define the morphology of the VM, for which electrophysiological data were
229 also recorded. To evaluate morphology of quadriceps and VM muscles, subjects were lying on
230 their backs on an auscultation table. Muscles anatomical cross-sectional area (ACSA) was
231 obtained using extended-field-of-view ultrasonography imaging (Mylab, Esaote, Genoa, Italy).
232 A 47 mm, 7.5 MHz linear array transducer was used to collect images at different muscle length
233 percentages. Three regions of interest were detected at 30%, 50% and 70% of femur length
234 (measured as the distance between the great trochanter and the mid-patellar point), where 0%
235 represents the mid-patellar point (distal part) and 100% the greater trochanter (proximal part).
236 An adjustable guide was used in each acquisition to keep the same transverse path (52). After
237 applying a generous amount of transmission gel on the participant's skin to improve the
238 acoustic contact, the transducer was moved slowly in the transverse plane from the medial
239 border of the VM to the lateral border of the vastus lateralis. During the transducer
240 displacement, we kept the pressure on the skin as constant as possible. Two scans were
241 obtained for each site.

242

243 *Force recordings*. Voluntary and electrically-evoked force of knee extensors was recorded using
244 a custom-made knee dynamometer fitted with a strain gauge. The hip and knee angles were
245 set at 90° (180° full extension). Subject's dominant leg was securely strapped to the custom-
246 made knee dynamometer, equipped with a load cell, at the ankle level. Subject was asked to
247 cross the arms on the chest and keep the head in a neutral position during the experiment
248 procedure. The force exerted by the subject was displayed in real-time on a computer screen
249 placed at 1 m in front of him. Because spinal excitability was further assessed during a weak
250 quadriceps contraction, a line representing 10% of the session MVC was added on the
251 computer screen once the MVC was determined. Force data were were recorded at a sampling
252 frequency of 1 kHz with the Spike2 software (version 8.01, Cambridge Electronics Design Ltd,
253 Cambridge, UK; RRID: SCR_000903) and stored for offline analysis with the LabChart software
254 (version 8, AD Instruments®, Australia; RRID: SCR_017551).
255
256 *Electromyographic recordings.* Electromyographic activity (EMG) from the dominant-leg VM
257 was recorded during the 10% MVC contractions with bipolar active-type Ag-AgCl electrodes
258 electrodes (44 x 22 mm, Ambu®). To minimize skin impedance (< 5 kΩ), the skin was shaved,
259 rubbed with an abrasive paste and cleaned with alcohol, before electrode positioning.
260 Electrodes were positioned 10 cm above the patella upper part and 4 cm medially with an inter-
261 electrode center-to-center distance of 20 mm. The EMG signals were amplified (gain = 1,000)
262 and bandpass filtered at 5 Hz to 5 kHz using a CED 1902 amplifier (Cambridge Electronics Design
263 Ltd, Cambridge, UK) and digitized with a CED Micro 1401 data acquisition unit (Cambridge
264 Electronics Design Ltd, Cambridge, UK). EMG data were recorded at a sampling frequency of
265 40 kHz with the Spike2 software (version 8.01, Cambridge Electronics Design Ltd, Cambridge,

266 UK; RRID: SCR_000903) and stored for offline analysis with the LabChart software (version 8,
267 AD Instruments®, Australia; RRID: SCR_017551).
268
269 *Nerve electrical stimulations.* Nerve electrical stimulations were delivered using a high-voltage
270 (400 V) constant-current stimulator (DS7AH model, Digitimer, Hertfordshire, UK). For maximal
271 voluntary activation assessment, electrical doublets (100 Hz, monophasic rectangular pulses, 1
272 ms width) were delivered through two stimulation electrodes (5 x 10 cm, Compex SA, Ecublens,
273 Switzerland) placed in the proximal and distal region of the knee extensors. Maximal electrical
274 stimulation intensity was determined as the intensity eliciting the maximal twitch amplitude.
275 For spinal excitability assessment, we delivered percutaneous electrical stimulations
276 (monophasic rectangular pulses, 1 ms width) in the femoral trigone over the femoral nerve to
277 evoke electrophysiological and mechanical responses of the VM muscle. The self-adhesive
278 anode (5 x 10 cm, Compex SA, Ecublens, Switzerland) was placed in the gluteal fossa. The
279 optimum stimulation site was located with a hand-held cathode stylus in order to obtain the
280 greatest H-reflex amplitude of the VM for the lowest stimulation intensity. Then, the self-
281 adhesive cathode electrode (7 mm diameter, Contrôle Graphique Medical, Brie-Compte-
282 Robert, France) was positioned and firmly fixed with a velcro elastic band.
283
284 *Mecanichal stimulations.* During T-reflex procedure, subjects were positioned as described
285 previously in *Force recordings* section. Twenty T-reflexes were elicited for each subject by
286 tapping the anterior face of the patellar tendon of the quadriceps with a manual reflex hammer
287 (neurological hammer, Babinsky model) at various intensities (53). The T-reflex on the VM EMG
288 signal and its associated mechanical twitch were recorded for each strike.
289

290 **Data analysis.**

291 *Ultrasonography.* Of the two scans obtained at each site, we analyzed the image with the best
292 quality. Quadriceps and VM ACSAs were measured by tracing the muscle contours with the
293 image analysis software "ImageJ" (https://imagej.net; RRID: SCR_003070). Two measurements
294 per parameter were performed on each image, and their average was considered. Quadriceps
295 ACSA (qACSA) was computed as the average value of the ACSAs recorded at 30, 50 and 70% of
296 the femur length for each subject at both LS0 and LS10. VM ACSA was calculated as the average
297 value of the ACSAs recorded at 30 and 50% of the femur length, due to technical issues in clearly
298 detecting the border between the VM and vastus intermedius at 70% of the femur length.

299

300 *Maximal force production*. The MVC was determined as the highest force value reached during
301 the 3 MVCs. The target of 10% MVC was calculated from the MVC of each session (i.e., LS0 or
302 LS10). The normalized force was calculated as the MVC divided by the quadriceps ACSA
303 (MVC/qACSA).

304

305 *Activation capacity*. The voluntary activation associated with the maximal force production and
306 the weak contraction (10% MVC) was quantified with the central activation ratio (CAR_{MVC} and
307 $CAR_{10\%}$ respectively) as follows (54):

308 $$CAR = \frac{Torque}{Torque + superimposed\ twitch}$$

309

310 where torque was either the MVC (CAR_{MVC}) or the 10% MVC ($CAR_{10\%}$). A higher CAR value
311 indicates an increase in muscle activation. It should be noted that, while the CAR_{MVC} was
312 calculated using maximal doublet stimulations (100 Hz) delivered during brief MVC, the $CAR_{10\%}$

313 was derived from the M-wave recruitment curve recorded during weak contractions at 10%
314 MVC using maximal single stimulations (see above *Spinal excitability* section). The ratio
315 between the $CAR_{10\%}$ and the force produced during the contraction at 10% MVC was calculated
316 to determine the motor unit activity required to achieve this specific target force. The root
317 mean square (RMS) of VM EMG activity was computed offline over a 500 ms period preceding
318 H_{MAX} and M_{MAX} stimulations during the weak contractions at 10% MVC, and subsequently
319 normalized to the M_{MAX} amplitude (RMS/M_{MAX}).
320
321 *Spinal excitability parameters.* One subject has been exempted from the spinal excitability
322 assessment because he did not tolerate electrical stimulations over the femoral nerve. Peak-
323 to-peak amplitude of both H-reflex and M-wave was measured and averaged for each
324 stimulation intensity. The highest amplitude of M-wave and H-reflex were labelled H_{MAX} and
325 M_{MAX} respectively. The H_{MAX}/M_{MAX} ratio was then calculated. In one subject it was not possible
326 to evoke the H-reflex at rest and was excluded from H_{MAX}-related parameters analysis at rest.
327 The amplitude of the M-wave accompanying H_{MAX} (M_{atHMAX}) was also measured and normalized
328 by M_{MAX} to verify that stimulation conditions were similar between sessions. The RMS of VM
329 EMG signal was measured over the 500 ms preceding each H_{MAX} (RMS_H) and M_{MAX} (RMS_M)
330 electrical stimulation during the contraction. We calculated the H_{MAX}/RMS_H ratio to assess the
331 influence of muscle activity background on spinal excitability (55,56). We also measured H_{MAX}
332 and M_{MAX} latencies at rest ($H_{LATENCY}$ and $M_{LATENCY}$) as the delay between the onset of stimulation
333 artefact and the upshot of electrophysiological response. While $M_{LATENCY}$ represents the time
334 required for conduction down the motor axons to the terminal branches and propagation
335 across the neuromuscular junction, $H_{LATENCY}$ reflects the time required for signal propagation
336 through the reflex arc, including the Ia afferent synaptic delay at the spinal level. Moreover, the

difference between $H_{LATENCY}$ and $M_{LATENCY}$ ($H\text{-}M_{LATENCY}$), representing an index of nerve conduction velocity through the spinal reflex loop, was computed (13). Then, these latencies were normalized by the subject's femur length (i.e., the distance between the greater trochanter and the lateral condyle). For one subject, the onset of the VM H_{MAX} was merged with the associated M-wave, thus this subject was excluded from H_{MAX} latency analysis. The amplitude of the mechanical twitch associated with both M_{MAX} (M_{TW}) and H_{MAX} at rest was measured. Since the M_{TW} represents the mechanical response of the muscle when the maximum amount of the motor units is recruited (57), its characteristics were analyzed. For each M_{TW}, we determined the contraction time (i.e., the time between twitch onset and maximal twitch value; CT), the rate of force development (i.e., the maximal value of the first derivative of the force signal using a 1 ms sliding window; RFD) and the half relaxation time (i.e., corresponding to the time needed to reach a 50% decrease of twitch force; HRT) before computing the averages of the four measurements. To assess the electromechanical efficiency, the M_{TW}/M_{MAX} was calculated. We analyzed the relationship between the amplitude of the T-reflex and the corresponding twitch since we did not have the complete T-reflex recruitment curve and therefore were not certain of obtaining the maximal T-wave response. The relationship between VM T-reflex amplitudes, expressed as a percentage of M_{MAX}, and the associated twitch amplitudes were scatter-plotted individually. The slope and the intercept of the linear regression of these relationships were determined for each subject and averaged at LS0 and LS10.

Statistical analysis. The normality of each dataset was assessed with the Shapiro-Wilk test. For all variables that passed normality test, Student's paired t-test was applied in order to investigate whether difference between LS0 and LS10 was present within the considered

361 parameters. When normality test was not passed, non-parametric Wilcoxon's signed-rank test
362 for paired comparison was used. Only for the RMS/M_{MAX} variable, a two-way repeated
363 measures ANOVA with *time* (LS0 *vs*. LS10) and *stimulation* (H_{MAX} *vs.* M_{MAX}) factors was
364 performed. If a significant effect was found, a Tukey's post-hoc analysis was conducted.
365 Correlations between MVC and qACSA as well as between MVC and CAR_{MVC} were analyzed
366 using repeated measures correlations (rmcorr R package, 51). Effect sizes (ES) were calculated
367 with Cohen's d and r respectively for parametric and non-parametric comparison (59). Small,
368 moderate and large effects are considered for Cohen's d ≥ 0.2, ≥ 0.5, ≥ 0.8, and Cohen's r ≥ 0.1,
369 ≥ 0.3, ≥ 0.5 respectively. Results are reported as mean ± standard deviation. Significance level
370 was set at $p < 0.05$ and the confidence interval at 95% ($CI_{95\%}$). Student's and Wilcoxon's tests
371 were performed using Statistica software (Statsoft, version 12, Tulsa, OK, USA; RRID:
372 SCR_014213) while Cohen's effect sizes were calculated with G*Power software (version
373 3.1.9.2, Universität Düsseldorf, Germany; RRID: SCR_013726). Repeated measures correlations
374 were performed with R (version 4.1.3, R Core Team, 2017; RRID: SCR_001905). Graphs were
375 made with GraphPad Prism software (version 8.4.0, GraphPad software Inc. San Diego, CA, USA;
376 RRID: SCR_002798).
377
378
379
380
381
382
383
384

385 RESULTS

386

387 *Muscle morphology, strength and activation capacity.*

388 The ACSA of the unloaded VM ($t_{(9)}$ = 4.042, $CI_{95\%}$ = [0.48 : 1.69], p = 0.003, ES = 0.426)
389 and quadriceps ($t_{(9)}$ = 3.444, $CI_{95\%}$ = [0.87: 4.19], p = 0.007, ES = 0.397) decreased (-7.8 and -4.4
390 %, respectively) after 10 days of ULLS (Table 1). The ACSA losses were accompanied by a 27.1
391 % reduction in knee extensors MVC ($t_{(9)}$ = 9.464, $CI_{95\%}$ = [17.01 : 27.70], p < 0.0001, ES = 2.196)
392 (Table 1). The normalized force (MVC/qACSA) was 23.7 % lower after ULLS ($t_{(9)}$ = 8.792, $CI_{95\%}$ =
393 [0.25 : 0.43], p = 0.0001, ES = 2.016) (Table 1).

394 Activation capacity as measured by CAR_{MVC} decreased by 3.0 % after ULLS ($t_{(9)}$ = 3.557,
395 $CI_{95\%}$ = [0.01 : 0.05], p = 0.006, ES = 1.175) (Figure 1) while the $CAR_{10\%}$ decreased by 15.0 % ($t_{(9)}$
396 = 4.011, $CI_{95\%}$ = [2.79 : 10.03], p = 0.003, ES = 0.997). The ratio between the CAR_{MVC} and the
397 MVC increases by 33.4 % following ULLS ($t_{(9)}$ = -6.839, $CI_{95\%}$ = [-0.01 : -0.00], p < 0.0001, ES =
398 1.880), while the ratio between the $CAR_{10\%}$ and the force produced at 10% of MVC increased
399 by 16.5 % ($t_{(9)}$ = -2.540, $CI_{95\%}$ = [-0.02 : -0.00], p = 0.032, ES = 0.869) (Table 1). Furthermore, the
400 normalized EMG activity (RMS/M_{MAX}) of the VM during the 10% MVC plateau declined by 34.2%
401 after ULLS (only *time* effect; $F_{(1,8)}$ = 8.482, p = 0.020, η_p^2 = 0.515 ; Tukey post-hoc : p = 0.020, ES
402 = 0.839) (Table 1). Based on repeated measures correlation analysis, it appears that MVC is
403 positively correlated to qACSA (r_{rm} (9) = 0.773, $CI_{95\%}$ = [0.22 : 0.95], p = 0.005) (Figure 2A) and
404 CAR_{MVC} (r_{rm} (9) = 0.624, $CI_{95\%}$ = [-0.07 : 0.91], p = 0.040) (Figure 2B). In other words, the higher
405 the qACSA or CAR_{MVC}, the greater the production of maximal force. However, MVC change was
406 not correlated to changes in CAR_{MVC} (r = -0.382, r^2 = 0.146, p = 0.276) and qACSA (r = 0.189, r^2
407 = 0.036, p = 0.600) (see Supplemental Figure S1).

408

409 *Muscle Contractile Properties*

410 Mechanical parameters associated with H_{MAX} and M_{MAX} stimulations at rest were also
411 compared between LS0 and LS10. The ULLS did not modify the twitch amplitude associated
412 with M_{MAX} (M_{TW}; $t_{(8)}$ = 1.593, $CI_{95\%}$ = [-0.69 : 3.75], *p* = 0.150, ES = 0.599) (Figure 3). Specifically,
413 the RFD of M_{TW} was not altered after 10 days of ULLS ($t_{(8)}$ = 1.685, $CI_{95\%}$ = [-0.01 : 0.09], *p* =
414 0.130, ES = 0.678), nor were the CT ($t_{(8)}$ = -1.159, $CI_{95\%}$ = [-14.61 : 4.84], *p* = 0.280, ES = 0.420)
415 and the HRT ($t_{(8)}$ = 0.165, $CI_{95\%}$ = [-15.05 : 17.36], *p* = 0.873, ES = 0.070). Also, the M_{TW}/M_{MAX}
416 ratio was not changed by the ULLS protocol (Z = 0.770, *p* = 0.441, ES = 0.257). Muscle contractile
417 properties obtained at rest are provided in Table 2. Regarding the twitch associated with H_{MAX},
418 its amplitude was not different between LS0 and LS10 ($t_{(7)}$ = 1.015, $CI_{95\%}$ = [-0.84 : 2.11], *p* =
419 0.344, ES = 0.253) (Figure 3).

420

421 *H-Reflex responses and M-wave*

422 While the H_{MAX} at rest was increased by 59.0% after 10 days of ULLS (Z = 2.100, *p* =
423 0.036, ES = 0.743), H_{MAX} obtained during muscle contractions at 10% MVC was not modified (Z
424 = 0.770, *p* = 0.441, ES = 0.257) (Table 3). It should be noted that the amplitude of the M-wave
425 associated with H_{MAX} (M_{atHMAX}) was not different between LS0 and LS10 either at rest (Z = 0.700,
426 *p* = 0.484, ES = 0.247) or during contraction (Z = 1.362, *p* = 0.173, ES = 0.454) (Table 3).
427 Therefore, the stimulation conditions at H_{MAX} may be considered stable, allowing the
428 comparison of spinal excitability features between the two time points. M_{MAX} did not differ
429 between LS0 and LS10 either at rest ($t_{(8)}$ = -0.258, $CI_{95\%}$ = [-1.83 : 1.46], *p* = 0.803, ES = 0.025)
430 or during 10% MVC contraction ($t_{(8)}$ = 1.345, $CI_{95\%}$ = [-0.42 : 1.59], *p* = 0.215, ES = 0.074) (Table
431 3). The ULLS increased the H_{MAX}/M_{MAX} at rest by 33.1 % (LS0 : 0.20 ± 0.15 *vs*. LS10 : 0.27 ± 0.15
432 ; $t_{(7)}$ = -2.557, $CI_{95\%}$ = [-0.13 : -0.01], *p* = 0.038, ES = 0.441) but had no impact on the ratio during

433 contraction at 10% MVC (LS0 : 0.34 ± 0.16 *vs.* LS10: 0.36 ± 0.17; $t_{(8)}$ = -1.947, $CI_{95\%}$ = [-0.04 :
434 0.00], *p* = 0.087, ES = 0.121) (Figure 4A). This result is also depicted in Figure 4B where
435 experimental traces of M_{MAX} and H_{MAX} obtained at rest, before and after ULLS, from one
436 representative subject, are presented. In order to overcome differences between sessions in
437 EMG activity during contractions at 10% MVC, we also normalized H_{MAX} by muscle activity
438 background preceding H_{MAX} stimulations (RMS_H), and we observed an increase of this ratio after
439 the ULLS period (+102.9 % ; $t_{(8)}$ = -2.480, $CI_{95\%}$ = [-74.00 : -2.70], *p* = 0.038, ES = 1.097) (Table 3
440 and Figure 4C).

441 Moreover, the delay between electrical stimulation and electrophysiological responses
442 was determined for the M_{MAX} and H_{MAX} waves. The latency of M_{MAX} ($M_{LATENCY}$) and H_{MAX} ($H_{LATENCY}$)
443 was not affected by ULLS ($M_{LATENCY}$: $t_{(8)}$ = -0.527, $CI_{95\%}$ = [-1.75 : 1.10], *p* = 0,613, ES = 0.192;
444 $H_{LATENCY}$: $t_{(6)}$ = -0.913, $CI_{95\%}$ = [-2.53 : 1.15], *p* = 0,396, ES = 0.134) (Table 3). The difference
445 between $H_{LATENCY}$ and $M_{LATENCY}$ (H-$M_{LATENCY}$) was not changed after 10 days of ULLS ($t_{(6)}$ = 0.289,
446 $CI_{95\%}$ = [-1.73 : 2.20], *p* = 0.782, ES = 0.049).

447 In addition, the ULLS did not modulate the relationship between the VM T-reflex and
448 the associated twitch. Indeed, the unloading period did not alter the slope ($t_{(5)}$ = -0.874, $CI_{95\%}$ =
449 [-0.31 : 0.15], p = 0.422, ES = 0.381) and the intercept ($t_{(5)}$ = -1.397, $CI_{95\%}$ = [-1.91 : 0.57], *p* =
450 0.221, ES = 0.946) of this linear regression (Table 3).

451

452

453

454

455

456

457 DISCUSSION

458 The present study aimed to investigate neural, contractile and morphological changes
459 associated with muscle weakness following 10 days of ULLS of the main antigravity muscle
460 group of the lower limbs. We showed that knee extensors' disuse led to a reduction in MVC
461 mainly explained by an impairment in central motor drive. For the first time, spinal excitability
462 was investigated in the quadriceps muscle group to deepen knowledge of neural adaptation to
463 unloading in antigravity muscles. Our main results show that spinal excitability was enhanced
464 at rest and during muscle contraction following 10 days of ULLS. These findings seem
465 particularly relevant as they provide new insights into the neurophysiological adjustments that
466 occur in a major antigravity muscle in response to human muscle disuse.

467

468 *Muscle morphology and function.*

469 The present investigation shows that 10 days of ULLS reduced the maximal force
470 developed by the knee extensors (-27 %, -2.7 %·day^{-1}). This alteration in MVC was associated
471 with a reduction in qACSA of 0.44 %·day^{-1} (-4.4 % in 10 days). In overall our results are consistent
472 with previous investigations that reported an impairment in force production capacity (11,14–
473 16,23,25,60) as well as a decrease in knee extensors size after ULLS (8,11,16,23)of the knee
474 extensors . As reported in the literature, muscle atrophy could be explained by decreases in
475 protein synthesis rate after lower limb muscle disuse (61,62) affecting the muscle protein
476 synthesis/breakdown balance (63) and thus, the myofibrillar content (64). The present study
477 corroborated, in agreement with earlier investigations (8,16,65–67), that strength loss, after
478 unloading, was greater than the rate of muscle size loss, therefore resulting in an alteration of
479 normalized force (-24 %). It is thought that muscular and tendinous alterations are involved,
480 such as a reduction in myosin content (16,68) and tendon stiffness (11), and an impairment of

481 the excitation-contraction coupling (67). Neural alterations, including a reduction in voluntary
482 activation and coordination of antagonistic muscles (69), could also be potential explanatory
483 factors contributing to the impairment of normalized force during muscle disuse. In the current
484 study, we observed an impairment of both maximal and submaximal central motor drive, as
485 revealed by the decrease in CAR_{MVC} (-3 %), $CAR_{10\%}$ (-15 %) and RMS/M_{MAX} (-34 %). Particularly,
486 a decrease in CAR_{MVC} was associated with a reduction in maximal force production capacity. In
487 addition, our data showed a rise in CAR_{MVC}/MVC and $CAR_{10\%}$/10% MVC ratios after the ULLS
488 protocol, suggesting an impairment in muscle electromechanical efficiency (i.e. the dissociation
489 between electrical and mechanical events of muscle function; 68) after knee extensors
490 unloading. Though our findings are consistent with some previous investigations on knee
491 extensors and plantar flexors after 21-30 days of limb suspension (23,29,60,71), some others
492 did not observed any change in maximal muscle activation, for the same muscle groups, after
493 10-30 days of limb unloading (11,13,16,25,30,72). Despite these conflicting results, strength
494 loss after unloading seems to be more related to alterations in neural than muscular factors
495 (13,73). As a matter of fact, among multiple predictive variables, including muscle ACSA, twitch
496 RFD and HRT or the amplitude of the H-reflex and M-wave for instance, it appears that central
497 activation is the most influential variable in mediating strength loss after muscle disuse (13).

498 Regarding mechanical twitch evoked by H_{MAX} stimulation at rest, no change was
499 reported following 10 days of ULLS in the current study. The present observation contrasts with
500 the result of Seynnes *et al.* (29) which reported a 40 and 54% increase in H_{MAX} twitch amplitude
501 for the plantar flexors after 14 and 23 days of ULLS respectively. This discrepancy might depend
502 on the muscle group investigated and the different duration of exposure to limb unloading. On
503 the other hand, in agreement with data from Campbell *et al.* (16) following 21 days of ULLS, the
504 current analysis, did not find any change in M_{TW}. However, it should be noted that there is no

505 consensus in the literature about the evolution of M_{TW} following muscle disuse, since studies
506 have shown that ULLS decreased (15,60,65) or even increased (30) the maximal twitch
507 amplitude on lower limb muscles. In the current study, the M_{TW} parameters (CT, RFD and HRT)
508 were unaltered after the knee extensors' unloading. These outcomes are in accordance with
509 the literature focusing on the muscle contractility of knee extensors following 21-30 days of
510 ULLS (16,30,60). Specifically, lower limb suspension did not result in the alteration of M_{TW} CT
511 (16), RFD (16,30,60) and HRT (16,30,60). It should be noted that we evoked a twitch to assess
512 muscular factors of knee extensors. The sensitivity of this index could not be sufficient to detect
513 changes in muscle contractility (74). Despite this, the present findings underline the
514 discrepancy between morphological (i.e. reduction in qACSA, maximal strength) and functional
515 (i.e. no change in quadriceps contractility) data already observed in the literature (8) and
516 suggest that short-term ULLS-induced strength loss is primarily attributed to neural factors.
517 Therefore, the present model of disuse does not seem to impair knee extensors' contractility
518 and relaxation processes, although it is effective in inducing strength loss through impairments
519 in muscle activation. Due to the relatively small number of data obtained for muscle
520 contractility outcomes (n = 9), further future studies will be required to confirm these findings.
521
522 *H-Reflex responses and M-wave*
523 As observed at rest by de Boer *et al.* (11) following 23 days of ULLS, our protocol did not
524 modify the maximal M-wave obtained at rest, and during a weak knee extensor contraction
525 (10% MVC). This result indicates that sarcolemmal excitability and potential action propagation
526 were not altered by limb unloading (75,76).
527 In the present study, unloading increased the spinal excitability of the VM at rest, as
528 revealed by the rise of 33% in H_{MAX}/M_{MAX}. Although there is no available data on knee extensors,

529 this finding is consistent with numerous earlier studies that have shown an increase in spinal
530 excitability of plantar flexors after 23-28 days of ULLS (7,8,12–14,29,30). Our results showed
531 that H_{MAX}/M_{MAX} obtained during a weak contraction (10% MVC) was not altered by ULLS. Since
532 presynaptic inhibitions are already partially released during voluntary contraction, the
533 possibility of observing an increase in the H_{MAX}/M_{MAX} ratio following the ULLS is limited in this
534 condition. However, it should be noted that the background EMG activity (i.e., the RMS of VM
535 EMG preceding H_{MAX} stimulation, RMS/M_{MAX}) was different between LS0 and LS10. To
536 overcome that limit, the H_{MAX} was normalized by the RMS_H (H_{MAX}/RMS_H). The latter parameter
537 appeared to be 2-fold greater after the 10 days of ULLS, meaning that, for a similar muscle
538 electrical activity, the amplitude of the maximal H-reflex was increased. In light of these results,
539 it appears that unloading induced an enhancement of H-reflex amplitude both at rest and
540 during contraction. As shown by Delwaide (34), this enhancement could be ascribed to an
541 increase in motoneuronal excitability and/or a decrease in presynaptic inhibition. Particularly,
542 the latter explanation has been confirmed in an immobilization model of disuse (28). In their
543 study, Lundbye-Jensen & Nielsen (28) showed that after immobilization of the left foot and
544 ankle joint by a cast for 14 days, the increase in *soleus* H-reflex results from decreased
545 presynaptic inhibition of the Ia afferents. More specifically, the authors reported a reduction in
546 the primary afferent depolarisation mechanism (i.e., a decrease in the inhibitory activity of a
547 spinal GABAergic interneuron) and a reduction in homosynaptic post-activation depression
548 (i.e., a decreased quantity of neurotransmitters available in the synapse following repeated
549 solicitation of the Ia afferents).

550 The present analysis showed that the decrease in muscle activation after a short period
551 of ULLS is accompanied by an increased spinal excitability. The latter could be viewed as a
552 compensatory adaptation, implemented by the nervous system to counterbalance the reduced

553 capacity to activate knee extensors. However, this adaptation is not sufficient to counteract the
554 entire cortical changes that occur during muscle disuse. Additionally, it seems that the increase
555 in the H-reflex following ULLS is not related to the activation capacity, as reported by Seynnes
556 *et al.* (30) on the plantar flexors after 24 days of limb unloading. Indeed, the authors did not
557 observe a significant relationship between relative changes in amplitude of resting H-reflex and
558 V-wave obtained during MVC over the ULLS period, indicating that the factors modulating the
559 former had a limited effect on the latter (30). The monosynaptic T-reflex of knee extensors,
560 evoked by patellar tendon striking, was also used as a supplementary tool to investigate the
561 modulation of spinal circuitry following knee extensors unloading. We observed no change in
562 the relationship between the T-reflex of the VM and the associated twitch, even if the spinal
563 excitability was enhanced. This result suggests that other factors that affect T-reflex, including
564 the mechanical properties of the tendon-aponeurosis and the sensitivity of the muscle spindle,
565 have probably been altered in such a way as to mask the effect of increased spinal excitability
566 on T-reflex (29). In contrast to our results, Seynnes *et al.* (29) reported an increase in the slope
567 and X-intercept of the T-reflex recruitment curve. Likely, the discrepancy between the present
568 and earlier findings may likely be ascribed to the muscle group investigated (knee extensors *vs.*
569 plantar flexors) and the ULLS duration (10 *vs.* 23 days). However, these interpretations should
570 be taken with caution because of the limited number of subjects (n = 6) that underwent the T-
571 reflex testing procedure during the present investigation.

572 In the current study, neither $H_{LATENCY}$, $M_{LATENCY}$ nor H-$M_{LATENCY}$ were affected by 10 days
573 of lower limb suspension, suggesting that nerve conduction velocity through the reflex arc and
574 synaptic delay at the spinal level were not impaired by unloading. This outcome is in contrast
575 to those of Clark *et al.* (13) and Seynnes *et al.* (30) who found an increase in soleus M_{MAX} and
576 H_{MAX} latencies after 28 and 24 days of ULLS respectively. More precisely, since the H-$M_{LATENCY}$,

577 an index of conduction velocity through the spinal reflex loop, was not changed in their studies
578 (13,30), authors primarily attributed the slowed conduction of H-reflex and M-wave responses
579 to the conduction velocity in the supply nerves, the branching axon terminals, and/or
580 transmission across the neuromuscular junction (77). No change in nerve conduction through
581 the reflex arc occurred after 10 days of ULLS for the lower limb muscles, and confirm similar
582 evidences from Manganotti *et al*. (78) who used the bed rest model during an identical period
583 of disuse. Further studies using ULLS protocols exceeding 10 days are needed to better
584 understand the modulation kinetics of nerve conduction in knee extensors.

585 In conclusion, the present study was the first to investigate the influence of short-
586 duration (10 days) ULLS on the neuromuscular function and spinal excitability of knee
587 extensors. The strength loss of knee extensors, observed after 10 days of lower limb unloading,
588 was mainly attributed to neural impairments, such as a reduction in central activation. To
589 compensate for reduced peripheral neurosensory inputs during the ULLS protocol, the spinal
590 excitability of the VM was enhanced at rest and during a weak knee extension (10% MVC) after
591 the ULLS protocol. These findings are paramount since they bring out new insights into the
592 neural adaptations that occur in the major antigravity muscle in response to muscle disuse.
593 Understanding the physiological adaptations during ULLS can guide the development of
594 effective interventions to improve overall health outcomes for individuals experiencing muscle
595 inactivity. For instance, using tendon vibrations (79,80) could be relevant to stimulate Ia
596 afferents in patients immobilised with a cast to improve spinal regulation of neural inputs (81)
597 and maintain efficient control of force production (82,83).

598

599

600

ACKNOWLEDGMENTS

The authors thank all volunteers who participated in the study.

GRANTS

The present study was funded by the Italian Space Agency (ASI), MARS-PRE, Project, n. DC-VUM-2017-006.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulate. They do not constitute endorsement by ACSM.

DATA AVAILABILITY

Data will be made available on reasonable request.

AUTHOR CONTRIBUTIONS

L.L., M.V.N., A.M., G.D., F.S. and G.S. conceived and designed research; L.L., F.S and G.S performed experiments; L.L. and F.S. analyzed data; L.L., M.V.N, A.M., G.D., F.S. and G.S. interpreted results of experiments; L.L. prepared figures; L.L. drafted manuscript; L.L., M.V.N, A.M., G.D., F.S. and G.S. edited and revised manuscript; L.L., M.V.N, A.M., G.D., F.S. and G.S. approved final version of manuscript.

SUPPLEMENTARY DATA

Supplementary data: https://doi.org/10.6084/m9.figshare.26779975

625 REFERENCES

626 1. Nash CE, Mickan SM, Del Mar CB, Glasziou PP. Resting injured limbs delays recovery: a
627 systematic review. J Fam Pract. 2004 Sep;53(9):706–12.

628 2. Lin CWC, Donkers NAJ, Refshauge KM, Beckenkamp PR, Khera K, Moseley AM.
629 Rehabilitation for ankle fractures in adults. Cochrane Database Syst Rev. 2012 Nov
630 14;11:CD005595.

631 3. Graham ZA, Lavin KM, O'Bryan SM, Thalacker-Mercer AE, Buford TW, Ford KM, et al.
632 Mechanisms of exercise as a preventative measure to muscle wasting. Am J Physiol Cell
633 Physiol. 2021 Jul 1;321(1):C40–57.

634 4. Evans WJ. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. Am J Clin Nutr. 2010
635 Apr;91(4):1123S-1127S.

636 5. Sarto F, Bottinelli R, Franchi MV, Porcelli S, Simunič B, Pišot R, et al. Pathophysiological
637 mechanisms of reduced physical activity: Insights from the human step reduction model
638 and animal analogues. Acta Physiologica. 2023;238(3):e13986.

639 6. Lee PHU, Chung M, Ren Z, Mair DB, Kim DH. Factors mediating spaceflight-induced
640 skeletal muscle atrophy. American Journal of Physiology-Cell Physiology. 2022
641 Mar;322(3):C567–80.

642 7. Hackney KJ, Ploutz-Snyder LL. Unilateral lower limb suspension: integrative physiological
643 knowledge from the past 20 years (1991-2011). Eur J Appl Physiol. 2012 Jan;112(1):9–22.

644 8. Campbell M, Varley-Campbell J, Fulford J, Taylor B, Mileva KN, Bowtell JL. Effect of
645 Immobilisation on Neuromuscular Function In Vivo in Humans: A Systematic Review.
646 Sports Med. 2019 Jun;49(6):931–50.

647 9. Tesch PA, Lundberg TR, Fernandez-Gonzalo R. Unilateral lower limb suspension: From
648 subject selection to "omic" responses. J Appl Physiol (1985). 2016 May 15;120(10):1207–
649 14.

650 10. Booth FW. Effect of limb immobilization on skeletal muscle. J Appl Physiol Respir Environ
651 Exerc Physiol. 1982 May;52(5):1113–8.

652 11. de Boer MD, Maganaris CN, Seynnes OR, Rennie MJ, Narici MV. Time course of muscular,
653 neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young
654 men. J Physiol. 2007 Sep 15;583(Pt 3):1079–91.

655 12. Clark BC. In vivo alterations in skeletal muscle form and function after disuse atrophy.
656 Med Sci Sports Exerc. 2009 Oct;41(10):1869–75.

657 13. Clark BC, Manini TM, Bolanowski SJ, Ploutz-Snyder LL. Adaptations in human
658 neuromuscular function following prolonged unweighting: II. Neurological properties and
659 motor imagery efficacy. J Appl Physiol (1985). 2006 Jul;101(1):264–72.

660 14. Clark BC, Pierce JR, Manini TM, Ploutz-Snyder LL. Effect of prolonged unweighting of
661 human skeletal muscle on neuromotor force control. Eur J Appl Physiol. 2007 Apr
662 12;100(1):53–62.

663 15. Horstman AM, de Ruiter CJ, van Duijnhoven NTL, Hopman MTE, de Haan A. Changes in
664 muscle contractile characteristics and jump height following 24 days of unilateral lower
665 limb suspension. Eur J Appl Physiol. 2012 Jan;112(1):135–44.

666 16. Campbell EL, Seynnes OR, Bottinelli R, McPhee JS, Atherton PJ, Jones DA, et al. Skeletal
667 muscle adaptations to physical inactivity and subsequent retraining in young men.
668 Biogerontology. 2013 Jun;14(3):247–59.

669 17. Cook SB, Kanaley JA, Ploutz-Snyder LL. Neuromuscular function following muscular
670 unloading and blood flow restricted exercise. Eur J Appl Physiol. 2014;114(7):1357–65.

671 18. Sarto F, Stashuk DW, Franchi MV, Monti E, Zampieri S, Valli G, et al. Effects of short-term
672 unloading and active recovery on human motor unit properties, neuromuscular junction
673 transmission and transcriptomic profile. The Journal of Physiology. 2022;600(21):4731–
674 51.

675 19. Dudley GA, Duvoisin MR, Adams GR, Meyer RA, Belew AH, Buchanan P. Adaptations to
676 unilateral lower limb suspension in humans. Aviat Space Environ Med. 1992
677 Aug;63(8):678–83.

678 20. Deschenes MR, Giles JA, McCoy RW, Volek JS, Gomez AL, Kraemer WJ. Neural factors
679 account for strength decrements observed after short-term muscle unloading. Am J
680 Physiol Regul Integr Comp Physiol. 2002 Feb;282(2):R578-583.

681 21. Deschenes MR, Holdren AN, McCoy RW. Adaptations to short-term muscle unloading in
682 young and aged men. Med Sci Sports Exerc. 2008 May;40(5):856–63.

683 22. Deschenes MR, McCoy RW, Mangis KA. Factors relating to gender specificity of unloading-
684 induced declines in strength. Muscle Nerve. 2012 Aug;46(2):210–7.

685 23. Schulze K, Gallagher P, Trappe S. Resistance training preserves skeletal muscle function
686 during unloading in humans. Med Sci Sports Exerc. 2002 Feb;34(2):303–13.

687 24. Tesch PA, Trieschmann JT, Ekberg A. Hypertrophy of chronically unloaded muscle
688 subjected to resistance exercise. J Appl Physiol (1985). 2004 Apr;96(4):1451–8.

689 25. Berg HE, Tesch PA. Changes in muscle function in response to 10 days of lower limb
690 unloading in humans. Acta Physiologica Scandinavica. 1996 May;157(1):63–70.

691 26. Duchateau J. Bed rest induces neural and contractile adaptations in triceps surae. Med Sci
692 Sports Exerc. 1995 Dec;27(12):1581–9.

693 27. Anderson J, Almeida-Silveira MI, Perot C. Reflex and muscular adaptations in rat soleus
694 muscle after hindlimb suspension. Journal of Experimental Biology. 1999 Oct
695 1;202(19):2701–7.

28. Lundbye-Jensen J, Nielsen JB. Immobilization induces changes in presynaptic control of group Ia afferents in healthy humans. J Physiol. 2008 Sep 1;586(Pt 17):4121–35.

29. Seynnes OR, Maffiuletti NA, Maganaris CN, de Boer MD, Pensini M, di Prampero PE, et al. Soleus T reflex modulation in response to spinal and tendinous adaptations to unilateral lower limb suspension in humans. Acta Physiol (Oxf). 2008 Nov;194(3):239–51.

30. Seynnes OR, Maffiuletti NA, Horstman AM, Narici MV. Increased H-reflex excitability is not accompanied by changes in neural drive following 24 days of unilateral lower limb suspension. Muscle Nerve. 2010 Nov;42(5):749–55.

31. Schieppati M. The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man. Prog Neurobiol. 1987;28(4):345–76.

32. Duchateau J, Enoka RM. Neural control of lengthening contractions. Lindstedt SL, Hoppeler HH, editors. Journal of Experimental Biology. 2016 Jan 1;219(2):197–204.

33. Grosprêtre S, Martin A. H reflex and spinal excitability: methodological considerations. J Neurophysiol. 2012 Mar;107(6):1649–54.

34. Delwaide P. Human monosynaptic reflexes and presynaptic inhibition. In: Desmedt JE, editor. Basel: Karger; 1973. p. 508–22. (New developments in electromyography and clinical neurophysiology; vol. 3).

35. Paillard J. [Electrophysiologic analysis and comparison in man of Hoffmann's reflex and myotatic reflex]. Pflugers Arch Gesamte Physiol Menschen Tiere. 1955;260(6):448–79.

36. Paillard J. Functional organization of afferent innervation of muscle studied in man by monosynaptic testing. Am J Phys Med. 1959 Dec;38:239–47.

37. Józsa L, Kvist M, Kannus P, Järvinen M. The effect of tenotomy and immobilization on muscle spindles and tendon organs of the rat calf muscles. A histochemical and morphometrical study. Acta Neuropathol. 1988;76(5):465–70.

38. Valli G, Sarto F, Casolo A, Del Vecchio A, Franchi MV, Narici MV, et al. Lower limb suspension induces threshold-specific alterations of motor units properties that are reversed by active recovery. Journal of Sport and Health Science. 2024 Mar;13(2):264–76.

39. Bleeker MWP, Hopman MTE, Rongen GA, Smits P. Unilateral lower limb suspension can cause deep venous thrombosis. Am J Physiol Regul Integr Comp Physiol. 2004 Jun;286(6):R1176-1177.

40. Roach REJ, Cannegieter SC, Lijfering WM. Differential risks in men and women for first and recurrent venous thrombosis: the role of genes and environment. J Thromb Haemost. 2014 Oct;12(10):1593–600.

41. Wanner M, Hartmann C, Pestoni G, Martin BW, Siegrist M, Martin-Diener E. Validation of the Global Physical Activity Questionnaire for self-administration in a European context. BMJ Open Sport & Exercise Medicine. 2017 May 1;3(1):e000206.

42. Guette M, Gondin J, Martin A. Time-of-day effect on the torque and neuromuscular properties of dominant and non-dominant quadriceps femoris. Chronobiol Int. 2005;22(3):541–58.

43. Douglas CM, Hesketh SJ, Esser KA. Time of Day and Muscle Strength: A Circadian Output? Physiology (Bethesda). 2021 Jan 1;36(1):44–51.

44. Hopkins JT, Ingersoll CD, Andrew Krause B, Edwards JE, Cordova ML. Effect of knee joint effusion on quadriceps and soleus motoneuron pool excitability: Medicine and Science in Sports and Exercise. 2001 Jan;123–6.

45. Zehr E. Considerations for use of the Hoffmann reflex in exercise studies. European journal of applied physiology. 2002 May 1;86:455–68.

46. Mau-Moeller A, Behrens M, Lindner T, Bader R, Bruhn S. Age-related changes in neuromuscular function of the quadriceps muscle in physically active adults. Journal of Electromyography and Kinesiology. 2013 Jun 1;23(3):640–8.

47. Doguet V, Jubeau M. Reliability of H-reflex in vastus lateralis and vastus medialis muscles during passive and active isometric conditions. Eur J Appl Physiol. 2014 Dec;114(12):2509–19.

48. Burke D, ADAMS RW, SKUSE NF. The effects of voluntary contraction on the H reflex of human limb muscles. Brain. 1989 Apr 1;112(2):417–33.

49. Knikou M. The H-reflex as a probe: pathways and pitfalls. J Neurosci Methods. 2008 Jun 15;171(1):1–12.

50. Funase K, Miles TS. Observations on the variability of the H reflex in human soleus. Muscle & Nerve. 1999;22(3):341–6.

51. McNeil CJ, Butler JE, Taylor JL, Gandevia SC. Testing the excitability of human motoneurons. Frontiers in Human Neuroscience [Internet]. 2013 [cited 2019 Dec 10];7. Available from: http://journal.frontiersin.org/article/10.3389/fnhum.2013.00152/abstract

52. Monti E, Franchi MV, Badiali F, Quinlan JI, Longo S, Narici MV. The Time-Course of Changes in Muscle Mass, Architecture and Power During 6 Weeks of Plyometric Training. Frontiers in Physiology [Internet]. 2020 [cited 2022 Nov 3];11. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2020.00946

53. Maffiuletti NA, Pensini M, Scaglioni G, Ferri A, Ballay Y, Martin A. Effect of electromyostimulation training on soleus and gastrocnemii H- and T-reflex properties. Eur J Appl Physiol. 2003 Nov;90(5–6):601–7.

54. Gandevia SC, Allen GM, Butler JE, Taylor JL. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol. 1996;8.

55. Theodosiadou A, Henry M, Duchateau J, Baudry S. Revisiting the use of Hoffmann reflex in motor control research on humans. Eur J Appl Physiol. 2023 Apr;123(4):695–710.

768 56. Palmieri-Smith R, Ingersoll C, Hoffman M. The Hoffmann Reflex: Methodologic
769 Considerations and Applications for Use in Sports Medicine and Athletic Training
770 Research. Journal of athletic training. 2004 Feb 1;39:268–77.

771 57. Nakagawa K, Fok KL, Masani K. Neuromuscular recruitment pattern in motor point
772 stimulation. Artificial Organs. 2023;47(3):537–46.

773 58. Bakdash JZ, Marusich LR. Repeated Measures Correlation. Front Psychol [Internet]. 2017
774 [cited 2022 Mar 31];0. Available from:
775 https://www.frontiersin.org/articles/10.3389/fpsyg.2017.00456/full

776 59. Fritz CO, Morris PE, Richler JJ. Effect size estimates: Current use, calculations, and
777 interpretation. Journal of Experimental Psychology: General. 2012;141(1):2–18.

778 60. Cook SB, Brown KA, Deruisseau K, Kanaley JA, Ploutz-Snyder LL. Skeletal muscle
779 adaptations following blood flow-restricted training during 30 days of muscular unloading.
780 J Appl Physiol (1985). 2010 Aug;109(2):341–9.

781 61. de Boer MD, Selby A, Atherton P, Smith K, Seynnes OR, Maganaris CN, et al. The temporal
782 responses of protein synthesis, gene expression and cell signalling in human quadriceps
783 muscle and patellar tendon to disuse. J Physiol. 2007 Nov 15;585(Pt 1):241–51.

784 62. Brook MS, Stokes T, Gorissen SHM, Bass JJ, McGlory C, Cegielski J, et al. Declines in muscle
785 protein synthesis account for short-term muscle disuse atrophy in humans in the absence
786 of increased muscle protein breakdown. J Cachexia Sarcopenia Muscle. 2022
787 Aug;13(4):2005–16.

788 63. Deane CS, Piasecki M, Atherton PJ. Skeletal muscle immobilisation-induced atrophy:
789 mechanistic insights from human studies. Clinical Science. 2024 Jun 19;138(12):741–56.

790 64. Brocca L, Longa E, Cannavino J, Seynnes O, De Vito G, McPhee J, et al. Human skeletal
791 muscle fibre contractile properties and proteomic profile: adaptations to 3 weeks of
792 unilateral lower limb suspension and active recovery: Impact of ULLS and active
793 rehabilitation on human muscle fibres. J Physiol. 2015 Dec 15;593(24):5361–85.

794 65. Clark BC, Fernhall B, Ploutz-Snyder LL. Adaptations in human neuromuscular function
795 following prolonged unweighting: I. Skeletal muscle contractile properties and applied
796 ischemia efficacy. J Appl Physiol (1985). 2006 Jul;101(1):256–63.

797 66. Demangel R, Treffel L, Py G, Brioche T, Pagano AF, Bareille MP, et al. Early structural and
798 functional signature of 3-day human skeletal muscle disuse using the dry immersion
799 model. J Physiol. 2017 Jul 1;595(13):4301–15.

800 67. Monti E, Reggiani C, Franchi MV, Toniolo L, Sandri M, Armani A, et al. Neuromuscular
801 junction instability and altered intracellular calcium handling as early determinants of
802 force loss during unloading in humans. The Journal of Physiology. 2021;599(12):3037–61.

68. D'Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canepari M, et al. The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. J Physiol. 2003 Oct 15;552(Pt 2):499–511.

69. Maganaris CN, Baltzopoulos V, Ball D, Sargeant AJ. In vivo specific tension of human skeletal muscle. J Appl Physiol (1985). 2001 Mar;90(3):865–72.

70. Turnsek G, Paravlic AH. Electromechanical efficiency index of skeletal muscle and its applicability: a systematic review. Front Bioeng Biotechnol. 2024;12:1398047.

71. Seynnes OR, Maganaris CN, de Boer MD, di Prampero PE, Narici MV. Early structural adaptations to unloading in the human calf muscles. Acta Physiol (Oxf). 2008 Jul;193(3):265–74.

72. Hotta N, Ishida K, Sato K, Koike T, Katayama K, Akima H. The effect of intense interval cycle-training on unloading-induced dysfunction and atrophy in the human calf muscle. J Physiol Anthropol. 2011;30(1):29–35.

73. Kawakami Y, Akima H, Kubo K, Muraoka Y, Hasegawa H, Kouzaki M, et al. Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise. European Journal of Applied Physiology. 2001 Feb 16;84(1–2):7–12.

74. Blinks JR, Rüdel R, Taylor SR. Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. J Physiol. 1978 Apr;277:291–323.

75. Fuglevand AJ, Zackowski KM, Huey KA, Enoka RM. Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces. J Physiol (Lond). 1993 Jan;460:549–72.

76. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008 Jan;88(1):287–332.

77. Kim BJ, Date ES, Park BK, Choi BY, Lee SH. Physiologic changes of compound muscle action potentials related to voluntary contraction and muscle length in carpal tunnel syndrome. J Electromyogr Kinesiol. 2005 Jun;15(3):275–81.

78. Manganotti P, Buoite Stella A, Ajcevic M, di Girolamo FG, Biolo G, Franchi MV, et al. Peripheral nerve adaptations to 10 days of horizontal bed rest in healthy young adult males. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2021 Sep;321(3):R495–503.

79. Nito M, Yoshimoto T, Hashizume W, Shindo M, Naito A. Vibration decreases the responsiveness of Ia afferents and spinal motoneurons in humans. Journal of Neurophysiology. 2021 Oct;126(4):1137–47.

80. Souron R, Baudry S, Millet GY, Lapole T. Vibration-induced depression in spinal loop excitability revisited. J Physiol. 2019 Nov;597(21):5179–93.

81. Rudomin P, Romo R, Mendell LM. Presynaptic Inhibition and Neural Control. Oxford University Press; 1998. 474 p.

840 82. Cook WA, Cangiano A. Presynaptic and postsynaptic inhibition of spinal motoneurons.
841 Journal of Neurophysiology. 1972 May;35(3):389–403.

842 83. Fink AJP, Croce KR, Huang ZJ, Abbott LF, Jessell TM, Azim E. Presynaptic inhibition of
843 spinal sensory feedback ensures smooth movement. Nature. 2014 May;509(7498):43–8.

844

845

846 FIGURE LEGENDS

847 **Figure 1.** Maximal force and corresponding central activation. The maximal voluntary
848 contraction (MVC) and corresponding central activation ratio (CAR_{MVC}) were individually scatter
849 plotted before (white circles) and after (grey circles) 10 days of unilateral lower limb suspension
850 (n = 10). Black (before limb suspension) and grey (after limb suspension) crosses represent the
851 mean of both scatter plots. ***$p < 0.001$ and $^{\$\$}p < 0.01$ express significant differences in MVC
852 and CAR_{MVC}, respectively, between before and after lower limb suspension.

853

854 **Figure 2.** Individual repeated measures correlations (rmcorr) between muscle function
855 parameters and maximal force production (n = 10). Rmcorr was plotted between qACSA and
856 MVC (A), and between CAR and MVC (B). Observations from the same participant are given the
857 same colour, the lines correspond to the rmcorr fit for each participant. MVC = maximal
858 voluntary contraction; qACSA = quadriceps cross-sectional area; CAR = central activation ratio.

859

860 **Figure 3.** Twitch amplitude associated with H_{MAX} and M_{MAX}. The twitch amplitude associated
861 with H_{MAX} (n = 8) and M_{MAX} (n = 9) was measured at rest before (white columns) and after (grey
862 columns) 10 days of unilateral lower limb suspension. Circles and lines represent individual data
863 evolution. H_{MAX} = maximal H-reflex; M_{MAX} = maximal M-wave.

864

865 **Figure 4.** Changes in spinal excitability after 10 days of ULLS. A. H_{MAX}/M_{MAX} ratio was calculated
866 before (white columns) and after (grey columns) 10 days of ULLS, either at rest (*n* = 8) and
867 during 10% MVC contractions (*n* = 9). Circles and lines represent individual data evolution. B.
868 H_{MAX} and M_{MAX} traces at rest, from a typical subject, before (red lines) and after (black lines)
869 ULLS. C. Representative data of VM EMG activity during the 10% MVC contraction with
870 associated H_{MAX} before (red line) and after (black line) ULLS from a typical subject. VM EMG =
871 electromyographic activity of vastus medialis; H_{MAX} = maximal H-reflex; M_{MAX} = maximal M-
872 wave. ULLS = unilateral lower limb suspension. *$p < 0.05$ express significant difference.

873

874 TABLES

875

876 Table 1. Muscle morphology, strength and activation capacity.

	BDC (n = 10)	LS9 (n = 10)	*p*-value
VM ACSA (cm^2)	16.34 ± 2.78	15.26 ± 2.26	0.003
qACSA (cm^2)	57.79 ± 6.54	55.26 ± 6.20	0.007
	LS0 *(n = 10)*	LS10 *(n = 10)*	*p*-value
MVC (kg)	82.54 ± 11.13	60.19 ± 9.13	< 0.0001
10% MVC (kg)	8.25 ± 1.11	6.02 ± 0.91	< 0.0001
MVC/qACSA ($kg \cdot cm^{-2}$)	1.43 ± 0.18	1.09 ± 0.16	0.0001
CAR_{MVC}	0.980 ± 0.013	0.950 ± 0.033	0.006
CAR_{MVC}/MVC (kg^{-1})	0.012 ± 0.002	0.016 ± 0.002	< 0.0001
$CAR_{10\%}$	0.428 ± 0.065	0.364 ± 0.063	0.003
$CAR_{10\%}$/10% MVC (kg^{-1})	0.052 ± 0.009	0.061 ± 0.011	0.032
RMS/M_{MAX} at 10% MVC ($x10^{-3}$)	9.39 ± 4.05	6.18 ± 3.54	0.020

877 All variables were measured before (LS0) and after (LS10) 10 days of unilateral lower limb
878 suspension, except ACSA measured the day of ULLS beginning (BDC) and the day before the
879 ULLS end (LS9). MVC = knee extensors maximal voluntary contraction; VM = vastus medialis
880 muscle; ACSA = muscle anatomical cross-sectional area; qACSA = quadriceps ACSA; CAR_{MVC} and

881 $CAR_{10\%}$ = central activation ratio calculated during a MVC and during a contraction at 10% MVC;

882 RMS = root mean square; M_{MAX} = maximal M-wave. Values are reported as mean ± standard

883 deviation.

884

885 Table 2. Mechanical parameters associated with M_{MAX} before and after ULLS.

	M_{MAX} stimulations		*p*-value
	LS0 *(n = 9)*	LS10 *(n = 9)*	
M_{TW}/M_{MAX} (kg/mV)	1.38 ± 1.07	1.33 ± 1.25	0.441
CT (ms)	82.44 ± 8.59	87.33 ± 14.03	0.280
RFD (kg/ms)	0.247 ± 0.060	0.210 ± 0.048	0.130
HRT (ms)	83.60 ± 15.15	82.44 ± 17.80	0.873

886 M_{MAX} = maximal M-wave; M_{TW} = twitch associated with M_{MAX}; CT = twitch contraction time; RFD

887 = twitch rate of force development; HRT = twitch half relaxation time; ULLS = unilateral lower

888 limb suspension; LS0 and LS10 = before and after 10 days of ULLS. Values are reported as mean

889 ± standard deviation.

890

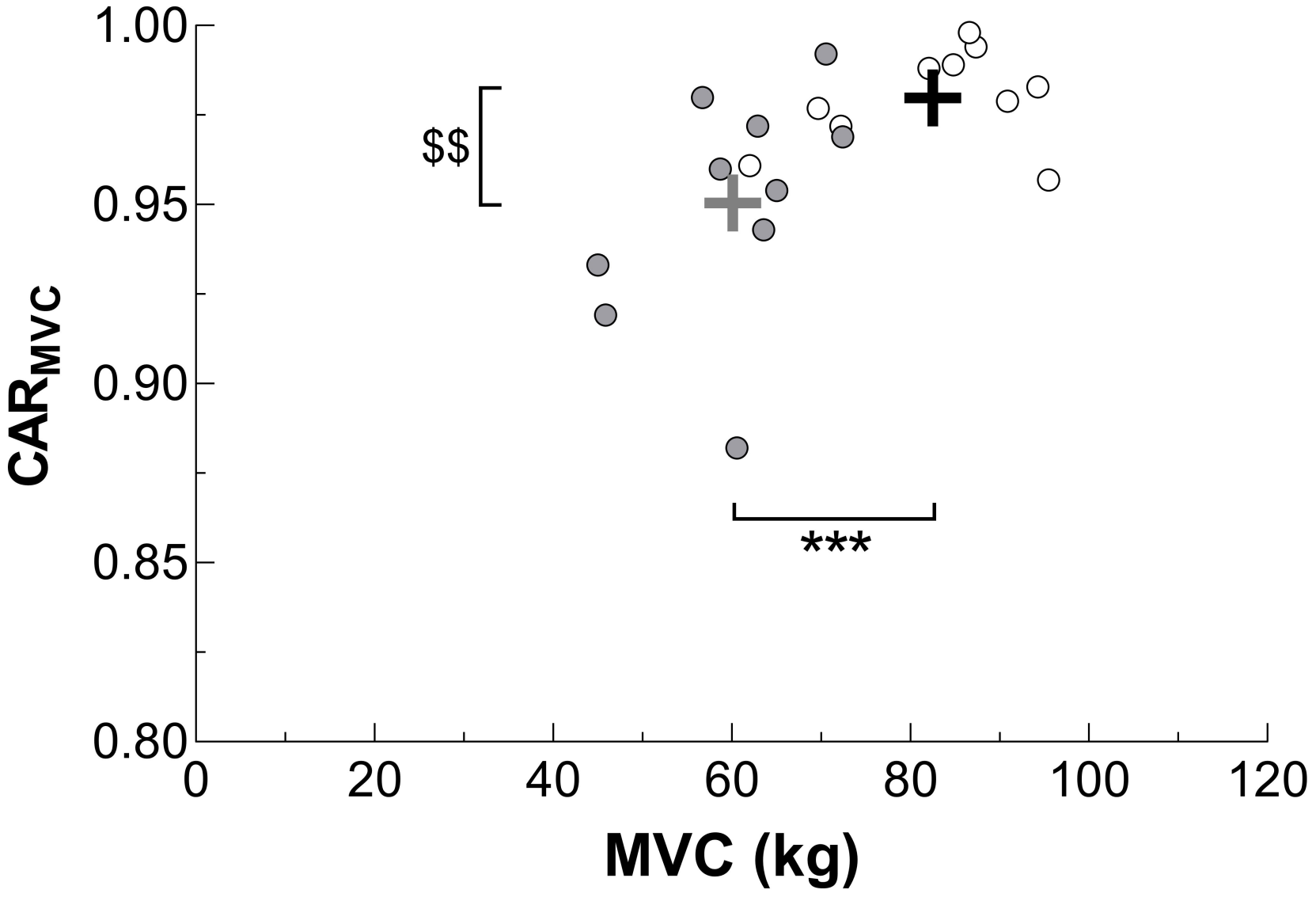
891 Table 3. Spinal excitability and nerve conduction parameters before and after 10 days of ULLS.

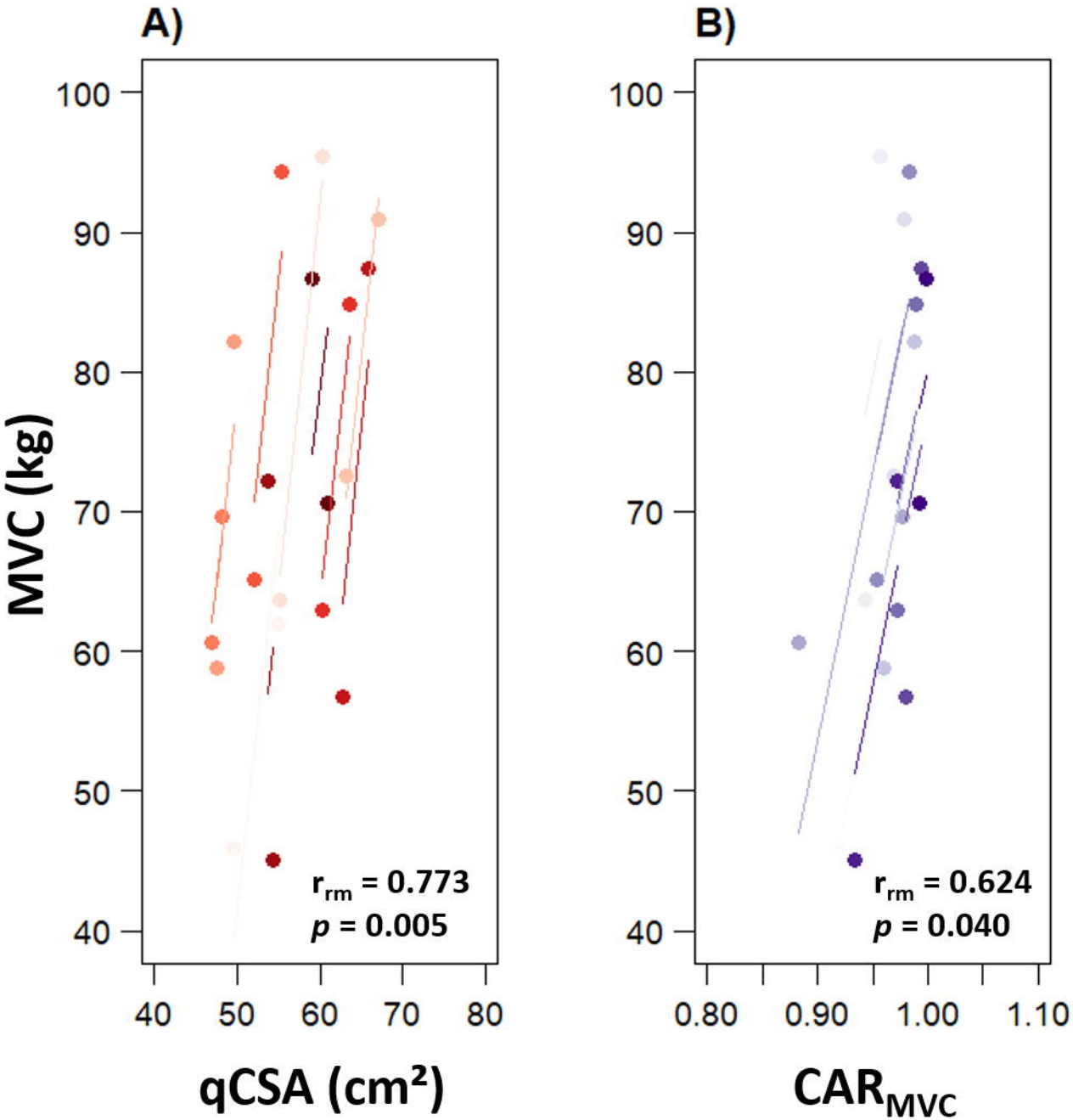
	Rest			Contraction		
	LS0	LS10	p-value	LS0	LS10	*p*-value
M_{atHMAX} (% M_{MAX})	8.39 ± 7.35	6.64 ± 8.65	0.484	9.02 ± 9.03	5.17 ± 4.63	0.173
H_{MAX} (mV)	2.22 ± 1.94	3.53 ± 3.25	0.036	4.29 ± 3.21	4.37 ± 3.44	0.441
M_{MAX} (mV)	13.12 ± 7.04	13.30 ± 7.41	0.803	13.48 ± 7.97	12.89 ± 7.91	0.215
H_{MAX}/RMS_H	-	-	-	37.27 ± 12.95	75.62 ± 47.69	0.038
$M_{LATENCY}$ (ms/m)	15.22 ± 1.60	15.74 ± 1.81	0.613	-	-	-
$H_{LATENCY}$ (ms/m)	48.04 ± 5.55	48.72 ± 5.55	0.396	-	-	-
H-$M_{LATENCY}$ (ms/m)	34.07 ± 5.34	33.84 ± 4.02	0.782	-	-	-
T-twitch slope	0.253 ± 0.096	0.331 ± 0.275	0.422	-	-	-
T-twitch intercept	0.876 ± 0.585	1.550 ± 0.807	0.221	-	-	-

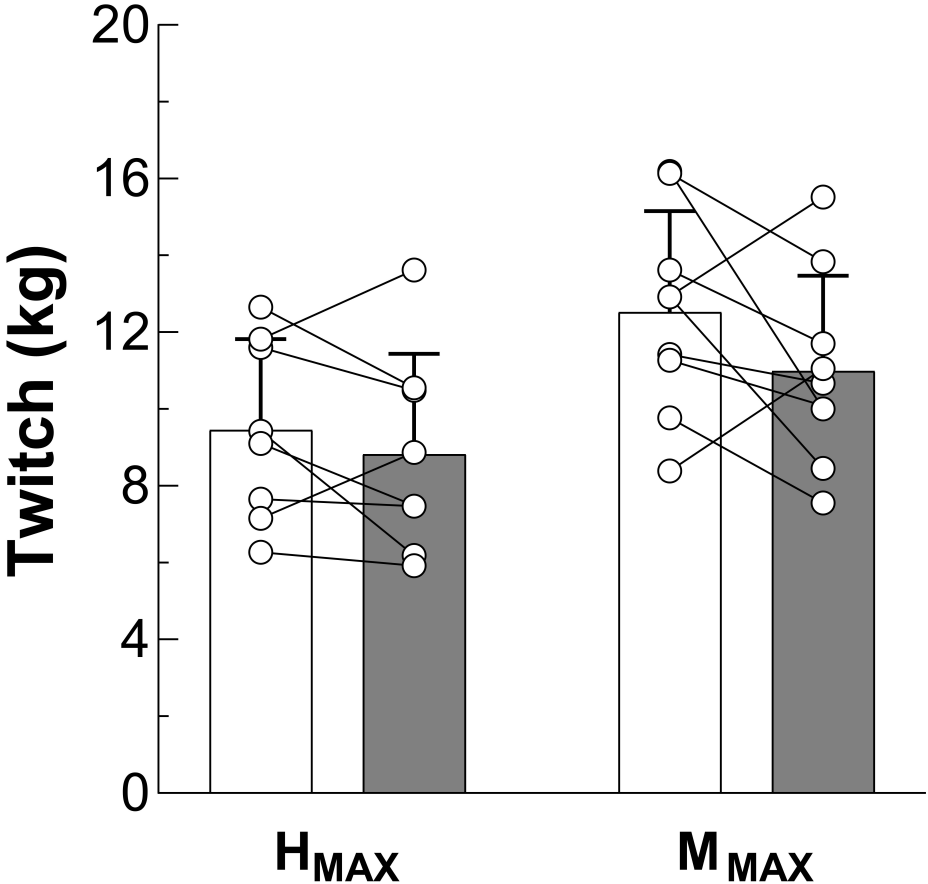
892 Data were obtained at rest and/or during 10% MVC contractions. For each parameter, the

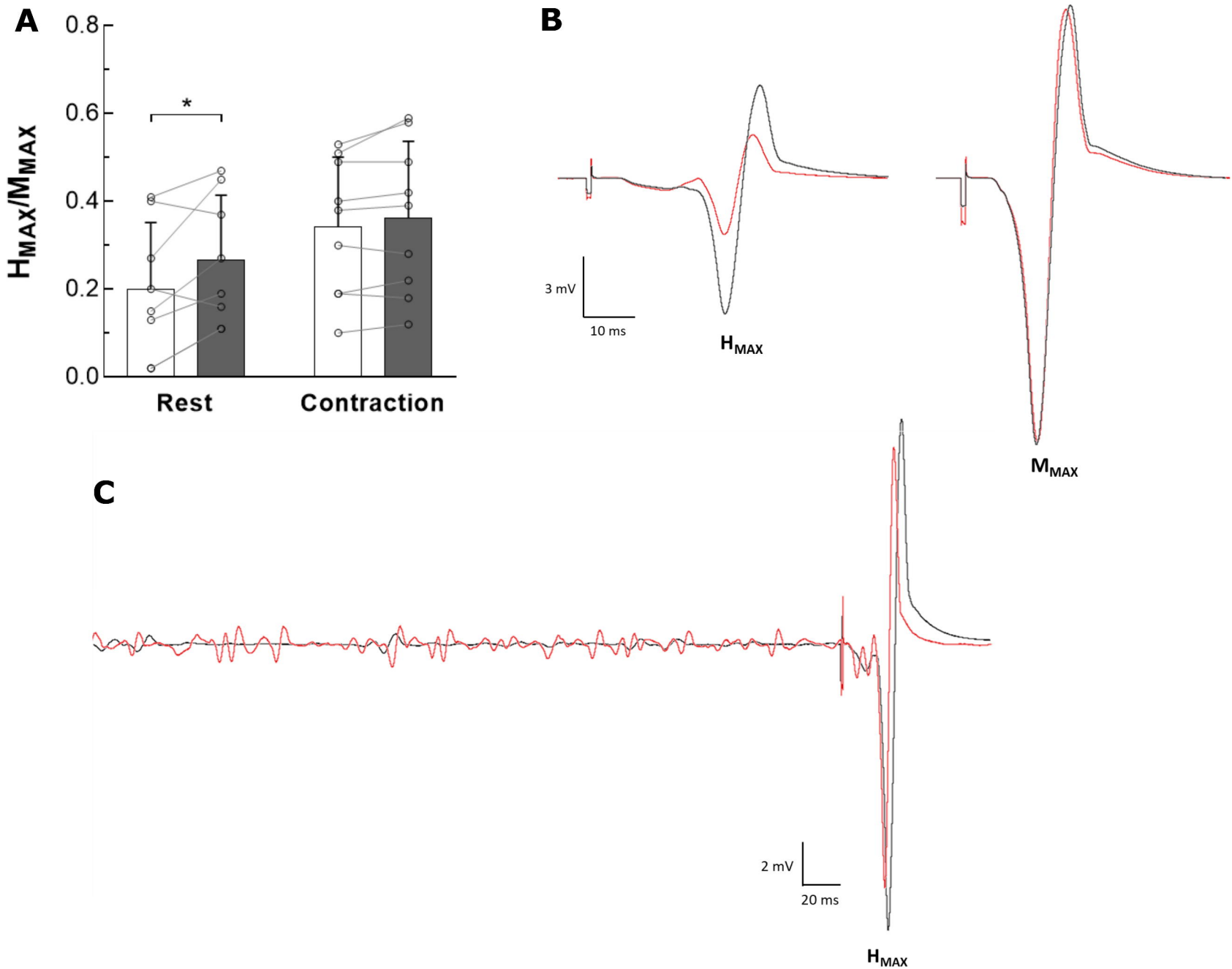
893 number of subjects was 9, except for M_{atHMAX} at rest (*n* = 8), $H_{LATENCY}$ and H-$M_{LATENCY}$ (*n* = 7), T-

twitch slope and intercept (n = 6). LS0 = before leg suspension; LS10 = after 10 days of leg suspension; H_{MAX} = maximal H-reflex; M_{MAX} = maximal M-wave ; M_{atHMAX} = M-wave associated with maximal H-reflex; $H_{LATENCY}$ and $M_{LATENCY}$ = H_{MAX} and M_{MAX} latency respectively; H-$M_{LATENCY}$ = difference between $H_{LATENCY}$ and $M_{LATENCY}$; T = T-reflex; twitch = T-reflex associated twitch; MVC = maximal voluntary contraction; ULLS = unilateral lower limb suspension. Values are reported as mean ± standard deviation. Please note that some parameters are obtained only in one condition (rest or contraction). *$p < 0.05$ and **$p < 0.01$ express significant difference from LS0 for the considered condition. Values are reported as mean ± standard deviation.


1.00
0.95
0.90
0.85
0.80
CARMVC
$$
***
0
20
40
60
80
100
120
MVC (kg)


A)
B)
MVC (kg)
100
90
80
70
60
50
40
40
50
60
70
80
qCSA (cm²)
r_rm = 0.773
p = 0.005
0.80
0.90
1.00
1.10
CAR_MVC
r_rm = 0.624
p = 0.040


Twitch (kg)
20
16
12
8
4
0
H MAX
M MAX


A
0.8
0.6
0.4
0.2
0.0
*
H_{MAX}/M_{MAX}
Rest
Contraction
B
3 mV
10 ms
H_{MAX}
M_{MAX}
C
2 mV
20 ms
H_{MAX}