RESEARCH ARTICLE

Peripheral nerve adaptations to 10 days of horizontal bed rest in healthy young adult males

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ABSTRACT

Space analogues, such as bed rest, are used to reproduce microgravity-induced morphological and physiological changes and can be used as clinical models of prolonged inactivity. Nevertheless, non-uniform decreases in muscle mass and function have been frequently reported, and peripheral nerve adaptations have been poorly studied, although some of these mechanisms may be explained. Ten young healthy males (18-33 y) underwent 10 days of horizontal bed rest. Peripheral neurophysiological assessments were performed bilaterally for the dominant (DL) and non-dominant upper and lower limbs (N-DL) on the 1st and 10th day of bed rest, including ultrasound of the median, deep peroneal (DPN) and common fibular (CFN) nerves, as well as a complete nerve conduction study (NCS) of the upper and lower limbs. Consistently reduced F-waves, suggesting peripheral nerve dysfunction, of both the peroneal (DL: p= 0.005, N-DL p= 0.013) and tibial nerves (DL: p= 0.037, N-DL p= 0.005) were found bilaterally, while no changes were observed in nerve ultrasound or other parameters of the NCS of both the upper and lower limbs were observed. In these young healthy males, only the F-waves, known to respond to postural changes, were significantly affected by short-term bed rest. These preliminary results suggest that during simulated microgravity, most changes occur at the muscle or central nervous system level. Since the assessment of F-waves is common in clinical neurophysiological examinations, caution should be used when testing individuals after prolonged immobility.

Keywords: bed rest, space, nerve conduction velocity, F wave, ultrasound, peripheral neurophysiology

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Key Points Summary

- Peripheral nerve morphological and conduction characteristics have been investigated before and after 10 days of bed rest in young healthy subjects
- Bed rest did not induce significant changes in the cross-sectional area of peripheral nerves
- Nerve conduction velocity and amplitude were not consistently affected by bed rest in both upper and lower limbs
- The H-reflex was only partially affected by bed rest
- The amplitude of F-waves in the lower limbs was significantly reduced after bed rest

Non-standard abbreviations

BDC1= baseline data collection one day before bed rest

BR = bed rest

BR0 = first day of bed rest

BR9 = last day of bed rest

CFB = Common Fibular Nerve

CSA = Cross Sectional Area

DL = Dominant Limb

DPN = Deep Peroneal Nerve

MEP = Motor Evoked Potentials

NCS = Nerve Conduction Study

NDL = Non-Dominant Limb

TMS = Transcranial Magnetic Stimulation

Introduction

Bed rest (BR) is a commonly accepted analogue of spaceflight [1, 2] used to observe and describe adaptations occurring in a simulated microgravity environment. Additionally, such model has been consistently used to understand the underlying mechanisms associated to the morphological and functional alterations following prolonged immobilization, as during hospitalization and in some clinical conditions [3, 4]. In particular, prolonged BR has been shown to induce metabolic adaptations, as anabolic resistance, which are key mechanisms to muscle atrophy that can be rapidly observable after few weeks of inactivity [5–7], with a 3% muscle thigh loss being reported after only 7 days of strict BR [8]. However, the magnitude of the loss in muscle strength and power after BR or limb immobilization is typically greater compared to the loss of muscle mass [9, 10]. As such, neurophysiological factors may explain the disproportionate loss of muscle function, as a previously described decrease in motor unit recruitment [11, 12] and may be associated to both central nervous system (CNS) and peripheral nervous system (PNS) adaptations.

Neurophysiological studies during BR have primarily focused on CNS mechanisms of adaptation, as neural plasticity [1, 13–15], including effects on to the number of synapses, degeneration of axonal terminals, alterations of the cerebral blood flow [16–18], as well as a significant reduction of leg excitability after only 2 weeks of BR (Roberts et al., 2010). Previous studies have indicated that reflex excitability is enhanced with unloading, as evidenced by a rise in Hoffmann (H)-reflex amplitude [19]. An increase in resting H-reflex amplitude with disuse has been observed in both human and animal models, and it has usually been attributed to a reduction in presynaptic inhibition (PSI) of Ia afferents and/or enhanced motoneuronal excitability. The possible impact of the unloading-induced increase in the resting H-reflex on the efferent neural drive is unknown. Some other studies, however, found reduced H-reflex during standing after 20-day BR [20, 21], which is again in contrast with the increased response during parabolic flight [22], suggesting that further studies are needed to differentiate the potential adaptation of H-reflex to the different microgravity analogues. Few studies assessing nerve morphological alterations and only preliminary electroneurographic findings suggesting decreased tibial nerve M response after 2 and 4 months of BR [23].

The data on peripheral nerve conduction during BR, both in experimental studies and in aerospace experiments, are lacking and inconclusive. A deeper understanding of peripheral nerve adaptations has also been encouraged [24], as it may help to discriminate the different mechanisms underlying BR induced weakness and reduced muscle power. Nerve conduction studies (NCS) are widely used in clinical practice and include a set of techniques to evaluate peripheral nerve (both motor and sensory) functional characteristics [25], as nerve conduction velocity, and electrically stimulated responses as the F-wave. For example, NCS is performed to support the diagnosis of intensive-care unit acquired weakness, including critical illness polyneuropathy and myopathy, which can be caused by immobilization, muscle

unloading, and mechanical ventilation [26]. Recently, nerve conduction velocity has been associated to morphological characteristics measured with ultrasonography (US) of the nerve, which has been proposed as a valid and cost-effective method to assess nerve morphological characteristics (as the cross-sectional area), and to detect and evaluate alterations due to trauma, inflammation, infection and compressive pathologies of the peripheral nerve [27, 28]. BR in healthy subjects represents a unique model to study neurophysiological changes induced by prolonged immobilization, without the common confounding factors that can be found in patients. In addition, alterations of peripheral nerve characteristics might help to discriminate between the different factors leading to reduced muscle strength and power [23, 24, 29].

Therefore, the aim of the present study was to measure the electroneurographic (NCS) and morphological (US) characteristics of the peripheral nerves of the upper and lower limbs in healthy adults at the first and last day of a 10-day BR protocol.

Materials & Methods

Participants

Ten healthy young adult men (aged 22.9±5 years, body mass 77.5±10 kg, height 1.81±0.04 m) were recruited to participate to the study. All participants passed physician examination with a routine blood and urine analysis. Exclusion criteria were: smoking; 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. Participants signed an informed consent form informing them of the purpose, procedures, and potential risk of the study. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and was part of a larger project entitled “Biological and Functional Markers for Precision Astronautical Biomedicine (MARS-PRE)” [9, 30]. It was approved by the National Medical Ethics Committee (ID: 0120-304/2019/9) and registered at ClinicalTrials.gov (NCT04081467).

Bed rest protocol

The study was conducted in a controlled medical environment at the General Hospital of Izola, Slovenia. Volunteers underwent 10 days of horizontal BR, were housed in standard air-conditioned hospital rooms, and were under constant video surveillance with 24-h medical supervision. For 10 days, participants performed daily activities lying in bed and received eucalorically controlled meals three times a day. Dietary energy requirements were designed on a group mean level by multiplying resting energy expenditure by a factor of 1.2 in BR period.

Magnetic Resonance Imaging protocol

Magnetic Resonance Imaging (MRI) data were acquired with a 3T scanner (Magnetom Skyra, Siemens, Erlangen, Germany) during the first day (BR0) and last day (BR9) of BR. Volunteers were instructed to stand still in a supine position on the patient bed after at least 8 hours of bed resting, allowing the stabilization of body fluid shifts. A turbo spin-echo T1-weighted data sets of both thighs were acquired in the transverse orientation. Acquisition parameters were as follows: Sequence: vibe, TR/TE: 7.8/3.69 ms, flip angle: 20°, field of view: 450 x 337.5 mm, voxel size: 0.9 x 0.9 x 6mm, no inter-slice gap, slice thickness: 6mm, readout-bandwidth 320 Hz/pixel (278 kHz). In the acquired scans, contours of the dominant limb Quadriceps Femoris muscle were digitized in randomized order using the OsiriX DICOM image analysis software (Version 11; Pixmeo Sarl, RRID:SCR_013618) as previously described [31]. Within the scan, quadriceps femoris cross-sectional areas contours were carried out every two axials; muscle volume was derived by summing a series of truncated cones between two axial images (i.e., ~20-25 images were evaluated per scan) [9].

Nerve ultrasound and conduction study

Participants were measured in the supine position at BR0 and BR9. Ultrasound examination was performed using a linear transducer (linear probe with sampling 14 MHz, Esaote portable ultrasound, Italy) in B-mode (gain 79%, PRS persistence 4, depth adjusted between 2-4 cm), keeping the participant supine in a resting position. All settings were maintained identical during both examinations on the same subject. Abundant gel was used, and the transducer was gently applied on the skin to reduce mechanical alterations. Conventional Nerve US is typically performed using probes with a frequency varying between 7 and 20 MHz, allowing imaging at a depth of 2-6 cm but with a resolution around 500 μm.

The median nerve was assessed at the location immediately proximal to the tunnel just before the nerve dips deeply to enter the carpal tunnel (2 cm proximal to the wrist), as previously described [32]. The deep peroneal nerve (DPN) was identified in a short axis approximately 3–4 cm proximal to the ankle (at the lateral malleolus level). At this location, the anterior tibial vessels serve as landmarks for finding the DPN [33]. Common fibular nerve (CFN) was assessed both above and below fibular head [34]. Quantitative analysis was provided by measuring the cross-sectional area (CSA) of the structures with the appropriate device tool. CSA (mm^2) of the whole nerve was determined using the trace function to outline the nerve along the inner border of the epineurium. For each nerve location, three images were taken. The same single ultrasonographer for all participants, experienced in neuromuscular ultrasound, performed all images acquisition and measurements and two raters independently traced the margin of the nerve. The mean of the three

measures for each rater, and the mean of the two raters was selected as the final value and entered the statistical analysis.

NCS is widely used in clinical practice and include a set of techniques to evaluate peripheral nerve (both motor and sensory) functional characteristics [25], as nerve conduction velocity, and electrically stimulated responses as the F-wave, which has been historically used to detect motor neuron excitability following deafferentation [35]. NCS was conducted bilaterally for all subjects, at the same time of the day and in the supine position by the same device (Electromyography Synergy, Synopo, Italy) and by the same experienced operator. Motor nerve conduction velocity and F and H responses were recorded from thenar (upper limb), extensor brevis digitorum and flexor digitorum (lower limb) using surface electrodes by stimulation of the median nerve, peroneal nerve distally (at the lateral malleolus level), below the head of the fibula, and above the head of the fibula, respectively for the upper and lower limb. F-wave responses were elicited by supramaximal stimulation of the median nerve once a second. Nine to ten F wave responses were collected at each recording session. For each set of 9-10 stimuli we measured the mean F wave peak-to-peak amplitude and the mean latency of the responses [36]. Sensory action potentials (SAP) for the II, III and IV finger of the hand were recorded using surface electrodes and stimulating the median nerve at wrist, while the sural nerve at the lower limb was recorded below the malleolus and stimulated at the calf. Intensity of the NCS stimulation is reported in the tables next to each nerve characteristics.

Quadriceps Femoris maximum isometric voluntary contraction

Peak Quadriceps Femoris force of the right leg (dominant limb) was assessed during an isometric maximum voluntary contraction at a 90° knee angle with hip fixed at 90° using a custom-made knee dynamometer fitted with a load cell (RS 206-0290). Participants were tested during the baseline data collection one day before BR (BDC1) and BR9. Prior to the testing session, participants were familiarized with the ergometer and the movement, being instructed to push as strong as they could. The volunteers were asked to perform three maximal contractions of 4 s duration with 60 seconds of rest between each contraction. The load-cell output was connected to an acquisition system (BIOPAC MP100, Biopac INC, Santa Barbara, USA), sampled at 2-kHz and analyzed using Ackqnowledge software (RRID:SCR_014279). The contraction with the highest force value was considered as real maximum voluntary contraction.

Statistical analysis

Continuous variables were tested for normality using the Shapiro-Wilk test. We summarized the data as means (standard deviation, SD). Data are presented and analyzed for the dominant limb (DL) and non-dominant limb (N-DL) independently. Reliability measures included the intraclass correlation coefficient (ICC) and the standard error of

measurement (SEM). Comparison between neurophysiological and muscle characteristics at the BR0 and BR9 was performed for all the participants with the Wilcoxon-signed rank test or with the paired-samples t-test. Effect size was determined with the Hedge's *g* value (95% CI: confidence intervals). A two-way repeated measures ANOVA (limb x time) was performed to assess differences between limbs, and a bivariate correlation analysis was performed using Spearman's correlation coefficient between the significant neurophysiological alterations and muscle changes in the DL. A significance level of $p < 0.05$ was selected.

Results

All ten young adult males initially selected and included in the BR successfully completed the entire study and were included in the final analysis. BR induced significant changes in muscle characteristics, including quadriceps force (-14.3 (9.8) %; p< 0.001, Hedge's *g* 0.646 95% CI 0.300–1.099), volume (-5.2 (4.0) %; p= 0.003, Hedge's *g* 0.265 95% CI 0.089–0.480), and CSA (-2.4 (2.0) %, p= 0.006, Hedge's *g* 0.120 95% CI -0.025–0.281) (**Table 1**). Assessment of reliability of the neurophysiological dependent variables showed ICC from 0.82 to 0.97. Nerve ultrasound revealed that all the participants had normal values in both upper and lower limbs, and no significant changes were found after BR (**Table 2**). NCS of the upper limbs suggested latency, amplitude or speed differences were not statistically significant (except for the latency of the median nerve distally in the dominant limb, that was found significantly increased after BR; p= 0.018, Hedge's *g* -0.254 95% CI -0.796–0.255), nor the F wave or the sensitive nerves (**Table 3**). In the lower limbs, NCS revealed no significant effects on the peroneal nerve latency, amplitude, or speed at any level of the measurement. However, significantly decreased F-wave amplitudes were found in both lower limbs in the peroneal nerve (DL– 40.0 (12.5) vs 25.0 (8.0) mV; p= 0.005, Hedge's *g* 1.306 95% CI 0.341–2.384; N-DL– 57.0 (38.5) vs 23.0 (12.5) mV; p= 0.013, Hedge's *g* 1.086 95% CI 0.156–2.173), and tibial nerve (DL– 54.0 (20.5) vs 35.0 (10.0) mV; p= 0.037, Hedge's *g* 1.077 95% CI 0.246–2.067; N-DL– 50.0 (18.5) vs 38.0 (16.0) mV; p= 0.005, Hedge's *g* 0.634 95% CI -0.153–1.508) (**Figure 1**). H-reflex was found altered only in the dominant limb, with a reduced latency after BR (32.7 (5.2) vs 30.0 (3.2) ms; p= 0.011, Hedge's *g* 0.571 95% CI -0.061–1.283). Sural sensitive nerve conduction showed different alterations between the limbs, with a reduced speed in the dominant limb (44.1 (16.2) vs 42.0 (7.0) m/s; p= 0.035, Hedge's *g* 0.153 95% CI -0.444–0.771), while the non-dominant limb was characterized by an increased latency (3.0 (1.0) vs 3.5 (0.5) ms; p= 0.042, Hedge's *g* -0.578 95% CI -1.174–-0.065) (**Table 4**). The two-way repeated measures ANOVA (limb x time) found only a significant time-effect in the above reported lower limbs outcomes, while no significant effects were found in the upper limbs. No significant correlations were found between the DL F-waves

amplitude changes and quadriceps force (peroneal n. p= 0.173; tibial n. p= 0.737), volume (peroneal n. p= 0.936; tibial n. p= 0.881) and CSA (peroneal n. p= 0.165; tibial n. p= 0.555).

Discussion

In this experimental model of short-term (10 days) BR in healthy males, characterized by changes in muscle force and mass consistent with previous studies [8, 10], we found a significant decrease in amplitude of F waves from peroneal and tibial nerves in lower limbs. In contrast, we observed no changes in conduction velocity (despite a surprisingly increased latency of the DL distal median nerve) or morphological aspects of peripheral nerves. Taken together, this data provide a deeper insight about the biomarkers of mechanical unloading that might help to explain the incongruent decline in muscle mass and function, as previously done for neuromuscular junction, excitation-contraction coupling, and supraspinal contributors [9, 10]. Few studies have investigated peripheral nerve adaptations to microgravity and its analogues [14, 20, 37]. In the present paper, several morphological and nerve conduction characteristics were described in healthy young adults during short-term BR.

The main finding of this study in young healthy males was a consistent decrease in the amplitude of F waves from tibial posterior nerve and peroneal nerve, which was significant after 10 days of BR (**Figure 2**). F-waves are low amplitude responses produced by artificial antidromic activation of motoneurons in electroneurography, and are largely used in standard neurophysiological examinations by measuring different parameters, such as latency, chronodispersion and amplitude [38]. Clinically, F-waves are used to investigate the focal proximal nerve dysfunction and are therefore a sensitive and reliable nerve conduction outcome to evaluate polyneuropathies and lumbosacral radiculopathies. In addition, F-waves can also provide a meaningful physiological window into spinal excitability with information on disorders of the central nervous system. The sensitivity to dynamic changes has been classically reported, showing an abnormal increase in F-wave chronodispersion in patients with spinal stenosis and neurogenic claudication after 3 minutes of standing [39], while 5 minutes of walking produced discernable and important increase in F wave latency and chronodispersion [40–42]. The observed decrease in F-waves amplitude without changes of latency after a 10-day BR was particularly intriguing, and some hypotheses might be suggested to explain this finding. Indeed, the F-wave is an artificial response which is present in motor axons even in deafferented roots [35], being produced by the conduction of mixed motor and afferent inputs at peripheral and spinal level. BR, which requires prolonged postural changes, might induce significant adaptations to afferent inputs either in the periphery or at the spinal level as documented in deafferented monkeys [35]. The Hoffmann reflex (H-reflex) is extensively used as both a research and clinical tool to investigate adaptive plasticity in spinal structures [43]. After a 20-day BR, the soleus H-reflex during standing was found significantly reduced after bed rest, without significant differences in motor evoked potentials (MEPs),

suggesting a strong inhibition of H-reflex and no adaptation of MEP in the soleus muscle [21]. Some of the potential mechanisms related to the altered H-reflex responses after simulated microgravity have been investigated suggesting a stretching of the spinal cord, cauda equina, nerve roots, and paraspinal tissues component [44]. In healthy individuals, H-reflex amplitude was found increased after 14 and 23 days of unilateral lower limb suspension [45]. In our findings, however, the H-reflex showed no significant alterations, although there was a tendency to reduced amplitude. Due to the paucity of studies [14, 46], these controversial results should be viewed with caution given the different inactivity models and duration.

In previous observations, the latency of the M response was found to be decreased after the end of 2 and 4 months of head-down BR study in young healthy subjects, and the decline was found to mostly last for 10 days [23]. A more in-depth analysis suggested a different time-dependent adaptation mechanism, with an initial increase in latency during the first weeks of BR, which was followed by the aforementioned decrease [23]. Present findings suggest a modest latency increase in the distal median nerve of the DL. Although due to the relatively small change it is not possible to give a certain explanation of the mechanisms behind this observation, and further studies are needed to better discuss this finding, a higher sensitivity of the dominant hand to the bed rest stimulus might be suspected, reflecting the initial increase in latency previously suggested [23]. In addition, we cannot exclude presence of fluid retention during BR with effect on mild nerve entrapment in sensible anatomical structures as the carpal tunnel [47, 48]. Some sensitive nerves action potentials parameters have been found partially changed after BR, despite results are inconsistent and require further investigations to confirm the observations. A recent study investigated somatosensory evoked potentials at cortical level after a 3-day dry immersion, suggesting shortened latencies of all central responses until P30 during the last day [49]. However, data are lacking about the somatosensory pathways in the peripheral nervous system [14], and both pain and thermal sensitivity have been found altered after BR [50–52]. Nevertheless, in our opinion and as reported in the present study, the absence of any significant changes in most conduction characteristics of motor and sensory potentials in peripheral nerve, as well as morphological nerve characteristics, further underpins the effects of inactivity on selective muscle sensitivity.

Effect of experimental immobilization-induced deafferentation on cortical and spinal excitability has been studied with transcranial magnetic stimulation (TMS) during prolonged BR, showing a decreased leg excitability in the immediate post BR period that lasted for around 2 weeks [53]. Decreased corticospinal excitability after BR has been reported also in TMS studies conducted on patients with leg/ankle fractures undergoing casting, with the duration of immobilization up to 60 weeks [54]. The absence of changes in conduction velocity of the main nerves of the lower limbs, the absence of changes in nerves morphological characteristics, as well as the non-significant correlations between the F-waves

amplitude changes and quadriceps muscle force and volume, suggest more central neurophysiological mechanisms to explain the unbalance between skeletal muscle mass loss and the reduction of muscle strength and power [6, 55, 56]. However, the reported effects on the F-waves should not be underestimated, and should be further investigated in other subjects, study durations and space (analogues) to better clarify the effects of postural changes and altered sensory afferences.

Perspectives and Significance

Mechanical unloading and prolonged disuse, such as during BR, induce several peripheral morphological and physiological adaptations, such as skeletal muscle atrophy with consequent loss of force production [57]. It is known that postural muscles (i.e. knee extensors and ankle plantar flexors) are more prone to atrophy than non-postural muscles in response to disuse and unloading, due to their role in standing and locomotion [58, 59]. Additionally, the duration of disuse of the lower limbs plays a key role in determining the amount of muscle atrophy, ranging from a relatively low but already significant decrease in thigh muscle volume (about 3%) after short term BR (7-day) [8], to a 10-12% decrement after longer BR (20 days) [60]. In older subjects, disuse (i.e. bed rest) further increases the detrimental effects of ageing on metabolism and muscle protein turnover [61], and may be common in frail individuals.

Physical inactivity or BR during hospitalization have been proposed as a primary factor contributing to functional decline in hospitalized patients [62, 63]. Indeed, the decrease in muscle strength associated with the unloading condition may have negative effects on gait descriptors and motor control of walking [64], leading to a decrease in walking economy associated with decreased independence and fatigue [65, 66]. Since the mechanisms underlying such adaptations might be complex and include a variety of integrated systems and tissues, describing the different adaptive profiles is necessary to develop appropriate and specific therapies and countermeasures.

The application of BR studies not only applies to “space physiology” but has been consistently translated to “Earth” as a model for inactivity and other adaptations that may occur in different pathological conditions. Peripheral neurophysiology is extensively studied in different diseases and is often performed as a clinical diagnostic test in individuals who exhibit abnormal sensory and motor functions, or in some cases in patients who are hospitalized and subject to prolonged BR, as in patients in intensive care units with systemic diseases, including during COVID-19 [67]. Understanding the physiological adaptations of the peripheral nervous system during prolonged BR in healthy subjects will help to better discriminate neurophysiological changes that occur due to a pathological condition or due to immobilization *per se*.

Conclusions

Neurophysiological adaptations to bed rest involve several mechanisms in both the peripheral nervous system and at the spinal level, with the main objective of compensating for reduced peripheral neuro-sensory inputs. Preliminary results from this study show that a 10-day bed rest in young persons had no effect on peripheral nerve conduction or morphology; however, the consistent decrease in F-waves amplitude suggests possible postural and reduced sensory inputs of bed rest, which may also occur in individuals in clinical settings.

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Disclosures

None of the authors has any conflicts of interests to declare.

Author Contributions

Conceptualization: PM, ABS, GB, UM, BS, MVN, RP; Methodology: PM, ABS, MA, GB, MF, EM; Investigation: PM, ABS, MF, EM; Formal Analysis: PM, ABS, MA, FGdG, GS; Software: ABS; Data Curation: PM; Writing - Original Draft: PM, ABS, FGdG, UM; Writing - Review and Editing: PM, ABS, MA, GB, MF, EM, GS, BS, MVN, RP. Project Administration: MVN and RP; Funding acquisition: MVN; Resources: PM, BS, MVN, RP; Supervision: PM, MVN, RP. All the authors read, revised, and approved the final version of the manuscript. All the authors agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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524 **Figure legends**

525 **Figure 1.** F-waves amplitude on the first (BR0) and last (BR9) day of bed rest in the dominant (DL) and non-dominant
526 (N-DL), measured on the peroneal nerve (A) and tibial nerve (B). Upper panels: reference site of the
527 measurement and stimulation. Lower panels: individual (n=10) BR0-BR9 lines with significance.

528 **Figure 2.** F-waves from the dominant limb tibial nerve on the first – BR0 (A) and last – BR9 (B) day of bed rest
529 measured in the same participant.

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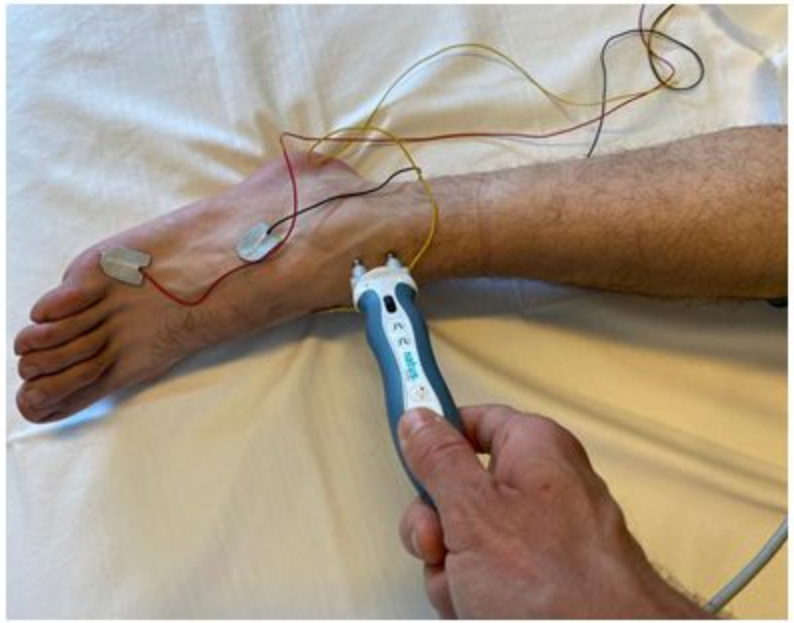
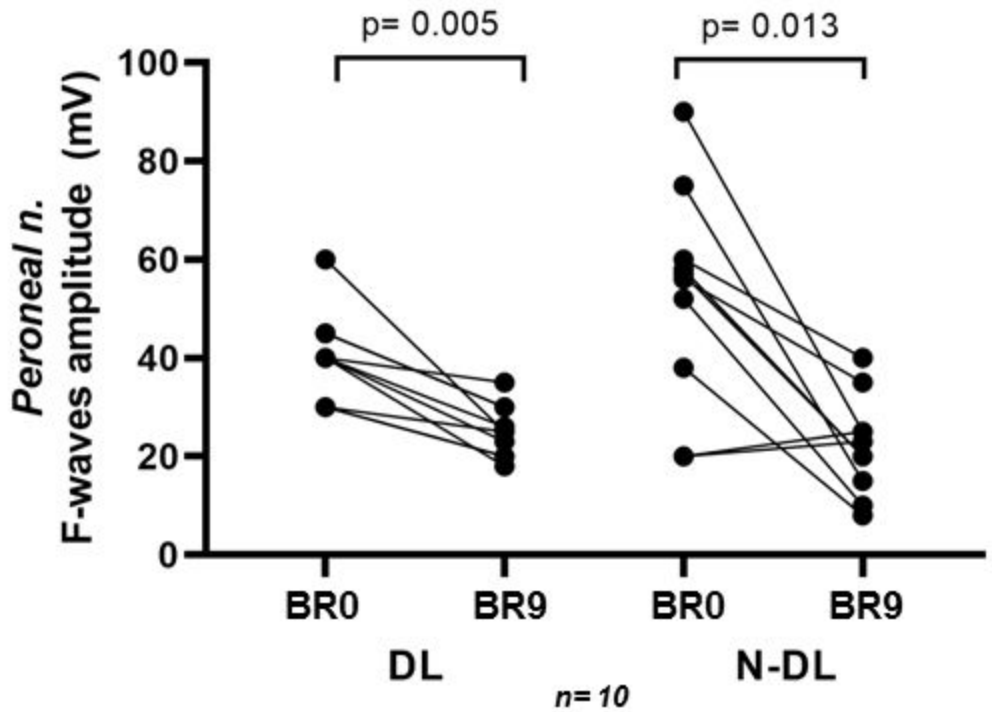
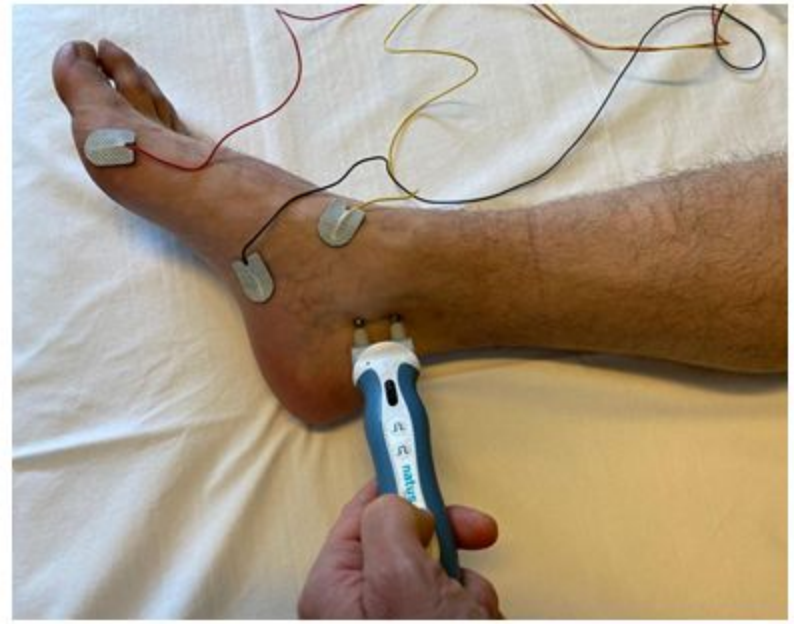
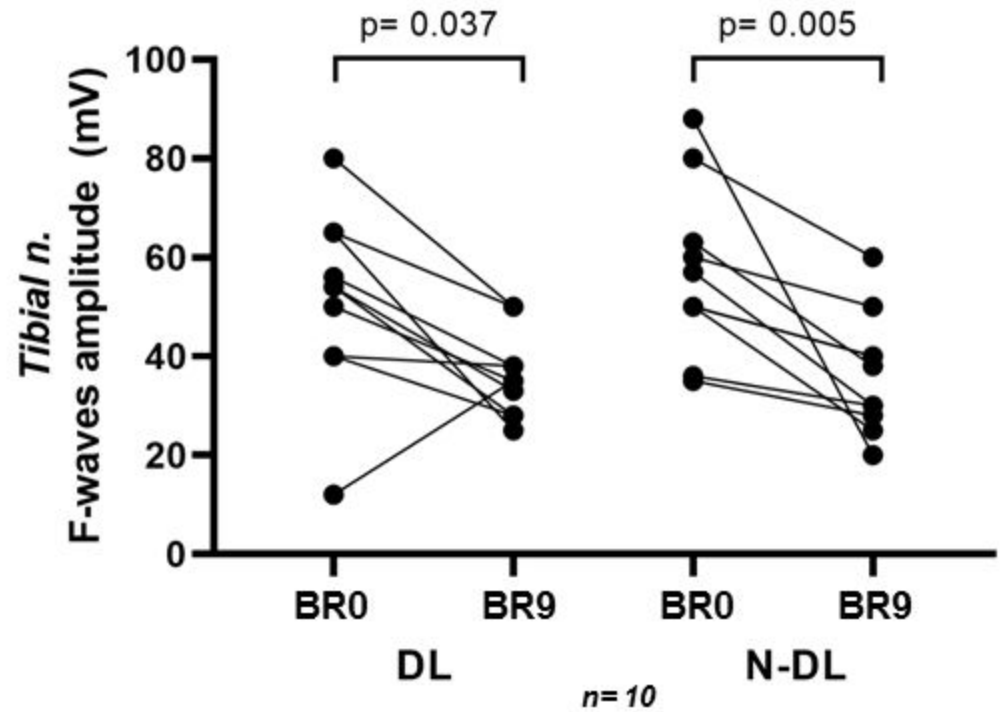
A




p= 0.005
p= 0.013
Peroneal n.
F-waves amplitude (mV)
100
80
60
40
20
0
BR0
BR9
BR0
BR9
DL
N-DL
n= 10

B




p= 0.037
p= 0.005
Tibial n.
F-waves amplitude (mV)
100
80
60
40
20
0
BR0
BR9
BR0
BR9
DL
N-DL
n= 10

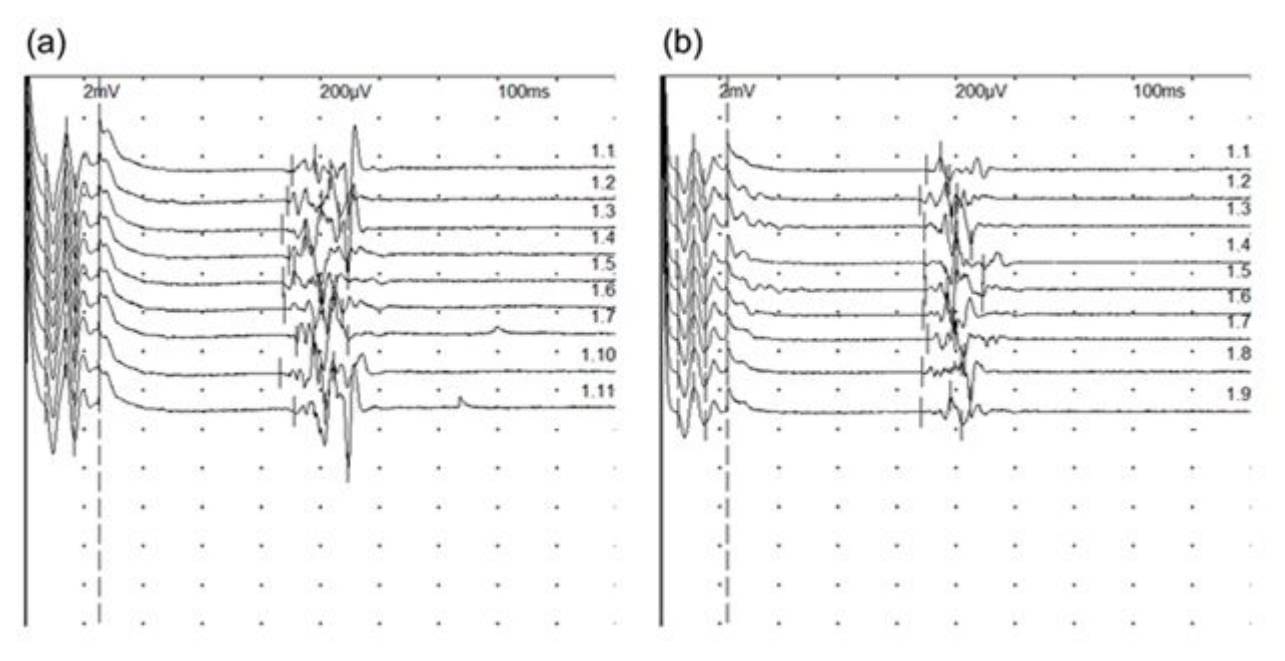

(a)
2mV
200μV
100ms
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.10
1.11
(b)
2mV
200μV
100ms
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9

Table 1: *Means (SD) of the quadriceps muscle force, volume, and CSA, at baseline data collection (BDC1) or first (BR0) and last day (BR9) of bed rest.*

Muscle	**DL**		**Sig.**
	BDC1/BR0	**BR9**	
Force (N)	777.3 (142.9)	662.8 (179.2)	**< 0.001**
Volume	2326.1 (428.3)	2203.5 (401.3)	**0.003**
CSA	69.9 (13.2)	68.2 (12.7)	**0.006**

Notes: CSA: cross-sectional area. Significance value for difference between the baseline (BDC1) and last (BR9) day of bed rest (for force), or between the first (BR0) and last (BR9) day of bed rest for volume and CSA. Ten young healthy males. Paired-samples t-test for differences between BDC1 and BR9. Bold values for $p < 0.05$**.**

Table 2: *Means (SD) of the selected nerves cross-sectional area (CSA) of the dominant (DL) and non-dominant (N-DL) limbs, at the first (BR0) and last day (BR9) of bed rest.*

Selected nerves CSA (mm^2)		**DL**		**Sig.**	**N-DL**		**Sig.**
		BR0	**BR9**		**BR0**	**BR9**	
Median – Wrist		7.9 (0.9)	8.2 (0.6)	0.434	8.2 (0.8)	8.0 (0.8)	0.343
	SEM	± 0.88	± 0.98		± 0.91	± 0.97	
DPN – Ankle		8.8 (2.1)	8.4 (0.8)	0.508	8.9 (0.9)	8.2 (0.8)	0.885
	SEM	± 0.94	± 0.92		± 0.93	± 0.99	
CFN – Below		9.0 (1.8)	9.3 (1.3)	0.591	9.3 (1.5)	9.5 (0.8)	0.726
	SEM	± 1.08	± 1.11		± 1.01	± 1.02	
CFN – Above		10.5 (1.4)	9.8 (0.9)	0.191	9.9 (1.4)	9.7 (1.8)	0.705
	SEM	± 1.02	± 1.00		± 0.99	± 1.06	

Notes: Median nerve distally (2 cm proximal to the wrist). CSA: cross-sectional area. Deep peroneal nerve (DPN) at the ankle (lateral malleolus level), common fibular nerve (CFN) below the head of the fibula, and above the head of the fibula. Ten young healthy males. Wilcoxon-signed rank test for differences between BR0 and BR9. SEM: standard error of measurement.

Table 3: *Means (SD) of the upper limbs NCS of the dominant (DL) and non-dominant (N-DL) limbs, at the first (BR0) and last day (BR9) of bed rest.*

NCS	DL		Sig.	N-DL		Sig.
	BR0	**BR9**		**BR0**	**BR9**	
Median n. – distal						
Latency (ms)	2.8 (0.8)	3.1 (1.3)	**0.018**	3.4 (3.4)	2.8 (1.0)	0.400
Amplitude (mV)	3.5 (4.5)	3.3 (7.0)	0.767	6.5 (3.6)	5.4 (3.0)	0.441
Speed (m/s)	56.6 (12.1)	54.3 (12.5)	0.110	57.0 (13.3)	56.0 (10.0)	0.173
Median n. – proximal						
Latency (ms)	7.5 (1.4)	8.0 (1.4)	0.342	7.4 (1.2)	7.7 (1.4)	0.293
Amplitude (mV)	3.4 (4.7)	3.5 (7.5)	0.953	6.5 (3.5)	4.5 (3.1)	0.327
Median n. – F wave						
mA	75.5 (34.0)	75.0 (34.0)		74.0 (48.5)	74.0 (48.5)	
Lat min (ms)	30.0 (7.2)	28.0 (4.2)	0.156	27.5 (2.5)	27.5 (4.0)	1.000
Lat max (ms)	32.5 (6.1)	30 (4.7)	0.080	29.5 (1.7)	29.0 (4.2)	0.176
Amplitude (mV)	31.5 (29.2)	46.0 (39.7)	0.241	36.5 (22.0)	54.0 (32.0)	0.325
Sensitive II						
mA	26.0 (13.2)	26.0 (13.2)		21.2 (6.4)	21.2 (6.4)	
Latency (ms)	3.2 (0.7)	1.9 (1.8)	0.173	2.2 (0.7)	2.4 (1.6)	0.207
Amplitude (μV)	43.0 (21.7)	47.0 (42.7)	0.314	42.5 (44.2)	65.0 (44.2)	0.858
Sensitive III						
mA	23.5 (7.5)	23.5 (7.5)		21.2 (6.4)	21.2 (6.4)	
Latency (ms)	3.0 (0.6)	2.2 (2.2)	0.441	2.8 (1.3)	2.4 (1.6)	0.327
Amplitude (μV)	50.0 (33.0)	48.0 (20.0)	0.678	47.0 (33.7)	50.5 (39.7)	0.678
Sensitive IV						
mA	23.5 (7.5)	23.5 (7.5)		21.2 (6.4)	21.2 (6.4)	
Latency (ms)	3.2 (0.8)	2.6 (1.8)	0.678	2.5 (1.6)	2.6 (1.5)	0.575
Amplitude (μV)	23.4 (4.8)	37.0 (50.2)	0.683	26.6 (24.9)	51.0 (41.0)	**0.015**

Notes: Nerve conduction study (NCS). Significance value for difference between the first (BR0) and last (BR9) day of bed rest. Median nerve distally at the wrist and proximal at the elbow. Ten young healthy males. Wilcoxon-signed rank test for differences between BR0 and BR9. Bold values for $p < 0.05$**.**

Table 4: *Means (SD) of the lower limbs NCS of the dominant (DL) and non-dominant (N-DL) limbs, at the first (BR0) and last day (BR9) of bed rest.*

NCS	DL		Sig.	N-DL		Sig.
	BR0	BR9		BR0	BR9	
Peroneal n. – distal						
Latency (ms)	4.5 (1.2)	5.2 (1.8)	0.374	5.2 (1.7)	4.1 (1.1)	0.050
Amplitude (mV)	6.6 (3.0)	5.0 (3.4)	0.953	6.0 (2.8)	5.1 (2.6)	0.441
Speed (m/s)	50.0 (8.3)	50.0 (3.0)	0.674	50.7 (6.9)	51.0 (7.5)	0.086
Peroneal n. – below						
Latency (ms)	12.0 (2.1)	12.3 (2.4)	0.508	12.5 (2.0)	12.1 (2.9)	0.343
Amplitude (mV)	7.5 (3.0)	6.6 (5.3)	0.594	6.5 (3.5)	5.0 (2.8)	0.214
Speed (m/s)	52.2 (9.7)	50.0 (13.0)	0.374	50.0 (17.8)	50.0 (3.0)	0.401
Peroneal n. – above						
Latency (ms)	13.5 (2.2)	13.4 (2.1)	1.000	13.6 (1.9)	13.3 (2.1)	0.374
Amplitude (mV)	7.2 (2.8)	6.4 (5.0)	0.594	6.7 (3.9)	4.8 (2.3)	0.093
Peroneal n. – F wave						
mA	63.0 (28.5)	63.0 (28.5)		50.0 (30.5)	50.0 (30.5)	
Lat min (ms)	50.0 (3.9)	50.0 (7.0)	0.779	48.0 (3.5)	50.0 (8.0)	0.134
Lat max (ms)	53.0 (4.0)	53.0 (5.0)	0.437	52.0 (4.5)	52.0 (7.5)	0.553
Amplitude (mV)	40.0 (12.5)	25.0 (8.0)	**0.005**	57.0 (38.5)	23.0 (12.5)	**0.013**
Tibial n. – F wave						
mA	32.0 (20.5)	32.0 (20.5)		55.0 (29.0)	55.0 (29.0)	
Lat min (ms)	51.0 (3.5)	50.0 (9.5)	0.331	52.0 (4.0)	52.0 (6.0)	0.153
Lat max (ms)	54.0 (4.5)	53.0 (10.0)	0.833	55.0 (5.0)	55.0 (5.5)	0.137
Amplitude (mV)	54.0 (20.5)	35.0 (10.0)	**0.037**	50.0 (18.5)	38.0 (16.0)	**0.005**
Soleus – H reflex						
mA	25.0 (21.0)	25.0 (21.0)		15.0 (16.8)	15.0 (16.8)	
Latency (ms)	32.7 (5.2)	30.0 (3.2)	**0.011**	31.0 (3.0)	29.7 (2.7)	0.086
Amplitude (mV)	1.0 (2.6)	1.0 (0.9)	0.065	1.5 (3.0)	0.9 (0.7)	0.075
Sural						
mA	25.0 (15.5)	25.0 (15.5)		30.0 (17.0)	30.0 (17.0)	
Latency (ms)	3.4 (1.4)	3.5 (0.7)	0.091	3.0 (1.0)	3.5 (0.5)	**0.042**
Amplitude (μV)	20.0 (15.8)	20.0 (5.5)	0.175	15.0 (19.0)	20.0 (6.0)	0.173
Speed (m/s)	44.1 (16.2)	42.0 (7.0)	**0.035**	50.0 (14.2)	43.0 (4.5)	0.083

Notes: Nerve conduction study (NCS). Significance value for difference between the first (BR0) and last (BR9) day of bed rest. Peroneal nerve distally (at the lateral malleolus level), below the head of the fibula, and above the head of the fibula. Ten young healthy males. Wilcoxon-signed rank test for differences between BR0 and BR9. Bold values for $p < 0.05$.