Title page

Title: Tensiomyography detects early hallmarks of bed-rest-induced atrophy before changes in muscle architecture

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Abbreviated title:

TMG-BEDREST

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Brief itemized list of how each author contributed to the study:

- Study concept and design: BŠ, JR, SL, RP, MN, HD
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- Analysis and interpretation of data: BŠ, KK, JR, CR, RP, MN, HD
- Drafting of the manuscript: BŠ, JR, SL, CR, ER, MN, HD
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Abstract

In young and older people skeletal muscle mass is reduced after as little as seven days of disuse. The declines in muscle mass after such short periods are of high clinical relevance, particularly in older people who show higher atrophy rate, and a slower, or even a complete lack of muscle mass recovery after disuse. Ten men (24.3± 2.6 years) underwent 35 days of 6° head-down tilt bed rest followed by 30 days of recovery. During bed rest, a neutral energy balance was maintained, with three weekly passive physiotherapy sessions to minimise muscle soreness and joint stiffness. All measurements were performed in a hospital at days 1-10 (BR1-BR10), day 16 (BR16), 28 (BR28) and 35 (BR35) of bed rest, and day 1 (R+1), 3 (R+3) and 30 (R+30) after reambulation. Vastus medialis obliquus (VMO), vastus medialis longus (VML) and biceps femoris (BF) thickness (d) and pennation angle (Θ) were assessed by ultrasonography, while twitch muscle belly displacement (Dm) and contraction time (Tc) were assessed with tensiomyography. After bed rest, d and Θ decreased by 13–17% in all muscles (P<.001) and had recovered at R+30. Dm was increased by 42.3–84.4% (P<.001) at BR35 and preceded the decrease in d by 7, 5 and 3 days in VMO, VML and BF, respectively. Tc increased only in BF (32.1%; P<.001) and was not recovered at R+30. Tensiomyography can detect early bed-rest-induced changes in muscle with higher sensitivity before overt architectural changes and atrophy can be detected.

Key words: tensiomyography, contraction time, skeletal muscle, rehabilitation, ageing

New & Noteworthy: Detection of early atrophic processes and irreversible adaptation to disuse is of high clinical relevance. Using Tensiomyography we detected early atrophic processes before overt architectural changes and atrophy can be detected using imaging technique. Furthermore, Tensiomyography detected irreversible changes of biceps femoris contraction time.

Introduction

Hospitalization due to injury or disease can lead to a period of forced inactivity. In those conditions skeletal muscle disuse is followed by atrophy, which in turn implies loss of contractile performance and metabolic dysregulation(30). Microgravity during space flight and the experimental models of disuse have a similar impact on muscle mass and function. Studies in young adults documented that skeletal muscle mass and strength are reduced after as little as seven days of spaceflight(20, 26) or bed rest(12) and continue to decline with the length of exposure(1). Declines in muscle mass and function after such short periods are of high clinical relevance to most patients who are, on average, hospitalized for <7 days(15). The disuse-induced loss of muscle mass is particularly relevant for elderly who show higher atrophy after 14-day bed rest and a much slower recovery or even complete lack of recovery for at least 14 days afterwards (33, 36). Therefore, there is a substantial need to develop methods to detect early stages of muscle atrophy related processes.

Evidences exist that muscle atrophy is not symmetrical throughout the muscle mass. Antigravity muscles show the greatest atrophy, and distal muscles atrophy more than proximal muscles(8). In addition, muscles with different functional roles across different joints and even muscles across the same joint may respond differently to unloading(3, 8). Rehabilitation programmes and assessments after any period of disuse should thus primarily focus on postural muscles and, at the same time, not overlook the non-postural muscles(8, 49).

At the human single muscle fibre level, evidence suggests that type I fibres depict stronger atrophy in bed rest than type II muscle fibres both after bed rest(6, 7) and spaceflight(17). Furthermore, there is a slow-to-fast myosin isoform transition after bed rest(31, 45) and spaceflight(50) that would result in faster contractile properties of the muscle, which will be accentuated by an increase in maximal shortening velocity of both type I and II muscle fibres after 17-day bed rest(48) and 17-day spaceflight(47). The latter effect seems reversed after

42(25) and 84 days bed rest(45), as well as after 180 days spaceflight(17). At the whole muscle level it has been reported that the time to peak twitch isometric tension of the triceps surae muscles was increased by 13%, indicating a slowing of the musculotendinous system after 120 days of bed rest(22). However, in this latter case, this was attributable to reduced tendon stiffness and increased muscle-tendon passive elasticity(23, 35), and thus not due to alterations in muscle contractile properties.

While ultrasound provides a reliable and non-invasive tool to follow structural changes of skeletal muscle during disuse, functional assessment of e.g. twitch torque requires specialised equipment and may not always be possible in bed ridden patient(21, 34, 38, 39, 41). To overcome this problem, relatively simple and low cost mechanomyographic methods were developed, where for instance Tensiomyography (TMG) allows for non-invasive and reliable(38, 44) estimation of contraction time (Tc), selectively in superficial muscle heads. This method can estimate the percentage of type I myosin heavy chain at least in the vastus lateralis (VL) muscle(39), and possibly also in other muscles. There is a clear distinction between results obtained from witch torque and TMG. For example, the Tc is 42.7% shorter when estimated from TMG than from twitch torque(21). This indirectly confirms that TMG gives better insights to the muscle contractility as it is less affected by the surrounding tissues(16, 21).

Using TMG, it was found that after 35 days of bed rest there was no change in Tc of the vastus medialis, but an increased Tc in gastrocnemius medialis muscle(34). The authors did, however, report that the TMG amplitude (Dm) was increased in both muscles, and that for gastrocnemius medialis the change in Dm was negatively correlated to the change in thickness (r=-.70). The Dm increase in both muscles in the abovementioned study may indicate a lower muscle resting tension and, possibly, decreased visco-elasticity(16).

While TMG detects changes after a prolonged disuse period(34), nothing is known about the possibility to adopt this method to follow initial and early changes in the adaptive response of muscle to disuse, before overt measurable atrophy. Therefore, the aim of our study was to assess 1) the time course of changes in muscle architecture and TMG parameters during 35 days bed rest and the following 30 days supervised recovery in young men, and 2) whether TMG is able to detect early changes that occur just after a few days of disuse.

Methods

Participants

Ten healthy men (age: 24.3±2.6 years, Table 1) with no history of neuromuscular or cardiovascular disorders participated in our study. The study was approved by the Slovenian National Medical Ethics Committee (approval number 72/06/08). All participants were fully informed about the study procedures and the possible health risks of study participation. Routine medical and laboratory analyses were performed to exclude participants with chronic diseases. None of the subjects regularly took any medication. From all participants written informed consent was obtained prior the study. All procedures were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its amendments.

<< Insert Table 1 >>

Experimental design

The bed rest study was conducted in the Orthopaedic hospital of Valdoltra under medical supervision. Participants arrived a week before the bed rest and were asked to visit the

147 laboratory on several occasions to become familiar with testing procedures. All baseline data
148 were collected (BDC) 1 day before the start of bed rest. After BDC, participants went through
149 35 days 6° head-down tilt bed rest followed by 30 days of supervised recovery. Subsequent
150 measurements were performed at days 1-10 (BR1-BR10), day 16 (BR16), 28 (BR28) and 35
151 (BR35) of bed rest, and day 1 (R+1), 3 (R+3) and 30 (R+30) after completion of bed rest. During
152 recovery, a fitness professional was available and all participants received written recovery
153 instructions. Recovery consisted of 12 sessions (3 sessions/week). Each session lasted about 60
154 minutes and consisted of a 10-min warm-up, 5 min active stretching, followed by 20 min
155 strength and balance exercises and 20 min aerobic exercises and a 5-min cool-down.

156 During bed rest, the participants received three weekly passive physiotherapy sessions to
157 minimise muscle soreness and joint stiffness. Each participant received a weight-maintaining
158 diet with an energy content of 1.4 and 1.2 times his resting energy expenditure, calculated using
159 the FAO/WHO equations(29), for the pre-bed rest and bed rest period, respectively(5). The diet
160 contained 60% of energy as carbohydrate, 25% as fat and 15% as protein. Six meals were
161 administered daily: 3 main meals (breakfast, lunch and dinner) and 3 snacks. Subjects were
162 required to consume all food served.

163

164 **Measurements**

165 **Ultrasonography**

166 Muscle architecture was determined at rest with B-mode ultrasonography (Mylab 25, 13-4
167 MHz, linear array transducer probe LA523, Esaote Biomedica, Geneva, Italy). Biceps femoris
168 (BF) scans were taken with the participant prone and with a knee angle set at 5° flexion with
169 foam pads. The BF measuring site was halfway between the ischial tuberosity and the posterior
170 knee joint fold, along the line of the BF long head. Vastus medialis obliquus (VMO) scans were

obtained supine at a knee angle set at 30° flexion with foam pads. The VMO measuring site was at the midpoint of the line from the patella to the VMO innervation point. The vastus medialis longus (VML) scans were obtained supine at 30° knee flexion at the midpoint of the line from the patella to the VML innervation point. The VMO and VML innervation points were detected using monophasic tetanic stimulation (impulse width 0.1 ms; frequency 10 Hz). To ensure that all subsequent ultrasound measurements were taken at the same anatomical location, the ultrasound probe was positioned in the midsagittal plane, orthogonal to the mediolateral axis, and its positioning was marked on acetate paper using moles and small angiomas as reference points.

For each muscle, three scans were obtained. Thickness (d in mm) and pennation angle (Θ in °) were measured using Matlab (Matlab, The MathWorks Inc., USA). In each scan, the fascicular path was determined as the interspaces between echoes coming from the perimysial tissue surrounding the fascicle. Muscle thickness was defined as the shortest distance between the deep and superficial aponeuroses. Pennation angle was defined as the angle between the fascicle pathway and the deep aponeurosis of the muscle. The average values for each architecture parameter of three scans was used for further analysis

Tensiomyography

Tensiomyography (TMG) was assessed in the same muscles at the same body positions and at the same measurement sites as ultrasound scans. TMG measurements were performed during electrically-evoked maximal isometric contractions. A single 1-ms maximal monophasic electrical impulse was used to elicit a twitch contraction that caused the muscle belly to oscillate. These oscillations were recorded using a sensitive digital displacement sensor (TMG-BMC Ltd., Ljubljana, Slovenia) that was placed on the surface of the skin at the measuring site

of the muscle of interest. Initially, the stimulation amplitude was set just above the threshold and then gradually increased until the amplitude of the radial twitch displacement (Dm in mm) increased no further. Electrical pulses ranged between 85 and 110 milliamperes at constant 30 volts. From two maximal twitch responses, also contraction time (Tc in ms) was calculated (Figure 1) as the time for the amplitude to increase from 10% to 90% of Dm (Figure 1)(39, 42). Furthermore, the velocity of radial displacement (V_r) was calculated by dividing .8·Dm with Tc(37).

<< Insert Figure 1 here >>

Statistics

SPSS (IBM Ltd., USA) software was used for all statistical analyses. All data in text and tables are presented as mean ± standard deviation, while in figures standard errors were used. Visual inspection and the Shapiro-Wilk test indicated that all data were normally distributed. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse-Geisser procedure. Main effects were studied with a General Linear Model repeated-measures ANOVA with time (BDC, BRi, R+j; where i = 1-10, 16, 28, 35 and j = 1, 3, 30) and muscle (VMO, VML, BF) as within factors. If a significant time x muscle interaction was found, the analysis was repeated with relative data representing percent change from BDC, to exclude any bias related to e.g. a difference in muscle thickness at BDC between muscles. Where significant time, muscle and interaction time x muscle effects were found, post-hoc analysis with Bonferroni corrections was used to locate the differences in time (p' = p/16; where 16 is the number of comparisons to the BDC value) for each muscle. Pearson regression analysis

was used to correlate changes during bed rest (Δ(BDC-BR35)) in Tc and Dm to changes in muscle architecture. Statistical significance was accepted at $p \leq .05$. The effect size for dependent variables was given as partial eta-squared (η^2).

Results

The variations in muscle structure as determined by ultrasonography and of muscle contractile function as measured with TMG are reported in Figure 2. Skeletal muscle thickness changed during the study ($P<.001$; $\eta^2=.865$; Figure 2A). Specifically, thickness declined progressively by 4.5% at BR7 ($P=.048$) to 15.2% at BR35 ($P<.001$), and recovered to BDC thickness at R+30 ($P=.22$). The absence of a time x muscle interaction ($P=.50$), indicates that the % changes in muscle thickness during bed rest and recovery did not differ significantly between muscles.

The time x muscle interaction ($P<.001$; $\eta^2=.938$) for Θ indicates that the changes in Θ over time differed between the three muscles. While the time course was qualitatively similar for the three muscles ($P<.001$; $\eta^2=.592$; Figure 2B), post-hoc analysis revealed that in the VMO Θ was first significantly decreased at BR6 (13.6%; $P=.033$), while in VML and BF it was already decreased at BR2 (5.5%; $P=.037$) and BR3 (7.4%; $P=.019$), respectively, interestingly at smaller decrease due to lower variance. In VMO and VML Θ had recovered to BDC at R+30 ($P>.05$) while in BF it was already recovered at R+3 ($P=.32$).

Two parameters characterize the TMG signal, the Dm and Tc, as well as the ratio between them, the V_r. The muscle x time interaction for Dm ($P<.001$; $\eta^2=.186$) indicates that the changes in Dm during the study ($P<.001$; $\eta^2=.782$; differed between the three muscles (Figure 2C). While the time course was qualitatively similar for the muscles, the magnitude of the rise in Dm was larger in the VML (84.4%) and BF (75.6%) than in the VMO (42.3%) at BR35 ($P=.013$;

η^2=.381). Dm increased already after BR1, BR4 and BR6 in VMO, VML and BF, respectively, and had returned to BDC at R+3 (P=.050).

The muscle x time interaction for Tc (P<.001; η^2=.255) indicates that the changes in Tc during the study (P<.001; η^2=.397; Figure 2D) differed between the three muscles. Post-hoc analysis revealed that Tc of the VMO did not change significantly during bed rest and recovery (P=.35), while the Tc of the VML (P<.001; η^2=.300) and BF (P<.001; η^2=.393) did change. We were unable to locate the difference with post-hoc tests in the VML. In the BF we found an increased Tc at BR7 (23.6% P=.043), being highest at R+1 (39.3%; P=.013). BF Tc did not return to the BDC value even at R+30 (26.4%; P=.041).

The muscle x time interaction for V_r (P<.001; η^2=.283) indicates that the changes in V_r during the study (P<.001; η^2=.733; Figure 2E) differed between the three muscles. We found differences in Vr at BDC (P=.017), where Vr was slowest in BF in comparison to VM muscles (P=.014). Furthermore, post-hoc analysis revealed that V_r of the VMO, VML and BF increased during bed rest for 40.7% (P<.001; η^2=.609) after BR9, for 74.6% (P<.001; η^2=.679) after BR6 and for 36.1% (P<.001; η^2=.418) after BR16, respectively. In all muscles V_r returned to BDC at R+1.

<< Insert Figure 2 here >>

The contractile parameters measured with TMG and the structural parameters measured with ultrasonography revealed correlations (Figure 3). Changes in muscle thickness and Dm between BDC and BR35 were negatively correlated. This negative correlation was significant in the BF (P=.001), but not in the VMO (P=.09) and VML (P=.06). There was also a positive correlation between Dm and Θ in VMO (P=.008) and VML (P=.050).

<< Insert Figure 3 here >>

Changes in Tc did not correlate significantly with changes in any of the architectural parameters (data not shown).

Discussion

Thirty-five days of 6° head-down bead rest induced a similar degree of atrophy (reduction in thickness) across all three muscles that had recovered 30 days after completion of bed rest. The atrophy was accompanied by a reduction in Θ that returned to baseline levels as soon as 3 days after cessation of bed rest. While the degree of atrophy became significant only after 7 days of bed rest, the increase in Dm was significant as soon as 1, 4 and 6 days after initiation of bed rest in the VMO, VML and BF, respectively. This suggests that Dm determined by TMG can be used to non-invasively and easily detect early hallmarks of the atrophy process, before overt atrophy was measurable by ultrasound.

After 35 days bed rest the muscle thickness was decreased by 16-23%, which is similar to the amount of atrophy seen in other studies(2, 4, 8, 28). In contrast to other studies(2, 4, 8, 28), we did not observe differences in the relative degree of atrophy between muscles. The discrepancy between these studies and ours may well be related to the range of muscles studied, where we assessed the bed-rest-induced changes only in the thigh, where others have compared the thigh muscles with muscles in the lower leg that atrophied more. It is likely that this difference in bed-rest-induced decreases in muscle mass between muscles is related to a larger reduction in

recruitment of lower leg than thigh muscles during bed rest. As expected, the atrophy was accompanied by a decline in Θ in all muscles as was previously also demonstrated(8).

Similar to a previous study we found that in all muscles Dm was increased by 35 days of bed rest though the increase in the present study was more pronounced than in that study using horizontal bed rest(34). This suggests that the fluid shift, away from the legs towards the head somehow affects the atrophy-induced increase in Dm. The fluid shift may also contribute to the observation that Dm was already elevated after as little as 24 hours of bed rest, before any overt architectural changes and muscle atrophy had taken place. In addition, the magnitude of Dm increase was between 42 and 84% after 35 days head-down tilt bed-rest and exceeded the atrophy that ranged between 16 and 23%. Another indicator that the fluid shift may play an important role in the increase in Dm with bed rest, is the almost instantaneous return of Dm after cessation of bed rest (at R+3), again before any significant architectural and muscle mass recovery had taken place (at R+30, except Θ in BF at R+3). How the fluid shift affects these changes is a matter of further research, but one might speculate that Dm may also be applicable to assess the hydration status of the muscle.

It is possible that fluid shifts out of the muscle may increase Dm by decreasing the viscoelasticity of the muscle-tendon tissue and decrease in muscle tone, resulting a larger bulging of the muscle in response to an identical electrical stimulus. The fluid shift from extremities to the chest can amount to a 4.4% decrease in extracellular fluid content that is particularly attributable to a loss of interstitial volume by 3% in parallel with a 12.3% reduction in plasma volume in just 4 days(19). After the 4^{th} day of bed rest plasma volume continues to decrease, but at a much slower rate(19). Later also intracellular fluid loss can occur that then parallels muscle atrophy(19).

Also dry immersion induces an increase in Dm and decrease muscle-tendon viscoelasticity(10, 24), that is at least partly attributable to a similar fluid shift away from the muscles. A decrease

313 in muscle tone, which occurs as early as after 1 day of dry immersion, may further contribute
314 to the increased Dm after 3 days of dry immersion(10) and after 20 days of bed rest(24). Such
315 changes have indeed been observed to translate into higher transversal muscle oscillations
316 during voluntary and electrically-evoked contractions(27).

317 Recent data show that merely a few days (e.g. 5-7 days) of disuse substantially reduce skeletal
318 muscle mass(11, 13), with a slower recovery rate in seniors than in young adults(33, 43). As a
319 consequence, it has been suggested that the accumulation of such short (<10 days), successive
320 periods of bed rest or immobilization during short-term illness or hospitalisation may contribute
321 to the loss of muscle mass and metabolic decline observed throughout life(14, 46). Given this
322 slow recovery in the older person, and being more prone to hospitalisation, it is important to
323 minimise, or even prevent, any atrophy. Identification of early functional and structural markers
324 of muscle deconditioning may help in designing adequate interventions to slow such atrophy
325 even before it becomes overt, and assess the success of an intervention to prevent atrophy(10).
326 Our data show that Dm may be such a functional marker, a parameter that can be determined
327 with high reproducibility(38, 44).

328 Bed rest did not induce a significant change in the Tc in the VMO, but did induce an increase
329 in the Tc in the VML and BF muscles. That observed increase was much more pronounced for
330 BF, where Tc also did not recover until 30 days after bed rest. Previously we found a positive
331 correlation between Tc and the MHC-I proportion in VL(39), and given that disuse is often
332 associated with a slow MHC-I to fast MHC-IIx transition, the correlation may not apply to
333 disused muscles, where for instance a decreased visco-elasticity may have a larger, and opposite
334 to, effect than the myosin heavy chain transition. However, the velocity of radial displacement
335 (Vr) increased in all muscles resulting from increased Dm and: (i) unchanged Tc in VMO, (ii)
336 slightly increased Tc in VML; and (iii) substantially increased Tc in BF. Although Vr should
337 not be paralleled to the contractile velocity of the whole muscle it is evident that Vr is sensitive

to muscle disuse as well as to assess peripheral fatigue after training(32) or peripheral arterial disease(18). However, further research is needed for the interpretation of Vr changes. Whatever the explanation, the data are analogous to the lower TMG-derived Tc in children and adults who participated regularly in sports(40, 42), or high-speed plyometric exercise(51). Indeed, when compared to previously published data, the magnitude of the increase in Tc after 35 days bed rest was comparable to or even more pronounced than that of sedentary childhood/adolescence or sedentary ageing (Table 2).

The increase in Tc in the BF following bed rest may have significant implications as it has been observed that a lower Tc correlated to higher vertical jump(51). The increase in Tc following bed rest in the BF, that was found also in seniors(42) may thus have significant clinical implications for the quality of life after hospitalisation. Therefore Tc of the BF is a parameter, like Dm, of special interest in assessing the efficacy of therapeutical interventions of people going through any kind of disuse, especially in the older population(33, 43).

<< Insert Table 2 >>

Conclusions/Relevance

In conclusion, our study showed that TMG can be used to detect early bed-rest-induced muscle dysfunction, before overt atrophy and atrophy-associated architectural changes can be detected with ultrasound. It remains to be seen whether such early changes are a result of the fluid shift away from muscle during head-down bed rest and/or is a reflection of structural bed-rest induced changes. Future studies in horizontal bed rest or unilateral limb suspension may shed light on the role of fluid shifts in TMG parameters. If no such changes are observed in such a model it is probably worthwhile to assess whether TMG can be used as clinical diagnostic tool

for atrophy and/or to assess the hydration status, something particularly important in older people and chronically ill patients where dehydration is related to sarcopenia and muscle weakness(9).

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Conflict of interest statement: There are no conflicts of interest.

373 **Table 1:** Anthropometric data of participants.

	BDC	BR35	R+30	P (η^2)
N	10			
Body height / m	1.78±6.5	1.78±6.5[1]	1.78±6.6	.92
Body mass / kg	75.3±9.3	72.2±8.7‡	74.8±8.2	<.001 (.709)
Fat mass / kg	15.8±3.6	15.7±3.2	14.4±2.6†	.003 (.470)
Body mass index / kg/m^2	23.7±1.9	22.7±1.7‡	23.6±1.7	<.001 (.700)

374 *Values are means ± SD; BDC: Before bed rest; BR35: 35 days bed rest; R+30: after 30 days*
375 *recovery; [1] body height was measured 12 hours after reambulation; * P<.05; † P<.01; ‡ P<.001*
376 *significantly different from BDC.*

377

378 **Table 2:** Biceps femoris contraction time of men: data from different populations/studies.

Population	N	Contraction time / ms	Reference
Children and adolescents			(40)
10 years – pooled	53	30.8 ± 5.0	
14 years – pooled	53	31.9 ± 6.3	
14 years – sedentary group	17	35.3 ± 9.1	
14 years – athletes	29	30.7 ± 6.1	
Adults (24 years)	10		This study
Before bed rest		28.3 ± 7.4	
After 35-day bed rest		36.1 ± 6.1	
After 30-day re-training		34.7 ± 6.9	
Adults, students (22 years)	20		(51)
Before plyometrics		30.6 ±7.7	
After 8 weeks of plyometrics		24.7 ± 5.9	
Adults and seniors			(42)
35-49 years – power master athletes	32	26.6 ± 7.0	
35-49 years – sedentary group	31	33.5 ± 7.0	
35-49 years – endurance master athletes	20	41.0 ± 8.5	
50-64 years – power master athletes	33	34.3 ± 8.9	
50-64 years – sedentary group	45	41.5 ± 11.4	
50-64 years – endurance master athletes	25	40.1 ± 6.5	
65+ years – power master athletes	35	38.9 ± 9.0	
65+ years – sedentary group	57	44.3 ± 9.2	
65+ years – endurance master athletes	31	53.4 ± 10.5	

379

380 *Values are means ± SD*

381

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Figure 1: Typical tensiomyographic response of the vastus medialis obliquus (left) and biceps femoris (right) at baseline (solid line) and after 35 days of bed rest (broken line).

Tc: contraction time defined as the time from 10% to 90% of the maximal displacement amplitude (Dm).

555 **Figure 2:** Changes in A) thickness (d), B) pennation angle (Θ), C) tensiomyographic
556 displacement (Dm), D) contraction time (Tc) and E) velocity of radial displacement (Vr) during
557 35 days bed rest and 30 days recovery in the Vastus medialis oblique (VMO), vastus medialis
558 longus (VML) and biceps femoris (BF).

559

560 *Values are means ± SE*

561

562 **Figure 3:** Pearson correlations between changes in tensiomyographic displacement (Dm) after
563 35 day bed rest and thickness (A-C) and pennation angle (Θ; D-F) in the vastus medialis
564 obliquus (VMO), vastus medialis longus (VML) and biceps femoris (BF).