RESEARCH ARTICLE | *Passive Properties of Muscle*

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

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Šimunič B, Koren K, Rittweger J, Lazzer S, Reggiani C, Rejc E, Pišot R, Narici M, Degens H. Tensiomyography detects early hallmarks of bed-rest-induced atrophy before changes in muscle architecture. *J Appl Physiol* 126: 815–822, 2019. First published January 24, 2019; doi:10.1152/japplphysiol.00880.2018.—In young and older people, skeletal muscle mass is reduced after as little as 7 days of disuse. The declines in muscle mass after such short periods are of high clinical relevance, particularly in older people who show a higher atrophy rate and a slower or even a complete lack of muscle mass recovery after disuse. Ten men (24.3 yr; SD 2.6) 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 minimize muscle soreness and joint stiffness. All measurements were performed in a hospital at *days 1–10*, *16*, *28*, and *35* of bed rest (BR1–BR10, BR16, BR28, and BR35, respectively) and *days 1*, *3*, and *30* after reambulation (R + 1, R + 3, and R + 30, respectively). Vastus medialis obliquus (VMO), vastus medialis longus (VML), and biceps femoris (BF) thickness (d) and pennation angle (Θ) were assessed by ultrasonography, whereas twitch muscle belly displacement (Dm) and contraction time (Tc) were assessed with tensiomyography (TMG). After bed rest, d and Θ decreased by 13–17% in all muscles ($P < 0.001$) and had recovered at R + 30. Dm was increased by 42.3–84.4% ($P < 0.001$) 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%; $P < 0.001$) and was not recovered at R + 30. TMG can detect early bed-rest-induced changes in muscle with higher sensitivity before overt architectural changes, and atrophy can be detected.

NEW & NOTEWORTHY Detection of early atrophic processes and irreversible adaptation to disuse are of high clinical relevance. With the use of tensiomyography (TMG), we detected early atrophic processes before overt architectural changes, and atrophy can be detected using imaging technique. Furthermore, TMG detected irreversible changes of biceps femoris contraction time.

aging; contraction time; rehabilitation; skeletal muscle; tensiomyography

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 7 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 the 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 afterward (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 programs and assessments after any period of disuse should thus primarily focus on postural muscles and at the same time, not overlook the nonpostural muscles (8, 49).

At the human single-muscle fiber level, evidence suggests that type I fibers depict stronger atrophy in bed rest than type II muscle fibers, 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 types I and II muscle fibers after 17-day bed rest (48) and 17-day spaceflight (47). The latter effect seems reversed after 42 (25) and 84 days of bed rest (45), as well as after 180 days of spaceflight (17). At the whole muscle level, it has been

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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 (24, 35) and thus not due to alterations in muscle contractile properties.

Whereas ultrasound provides a reliable and noninvasive tool to follow structural changes of skeletal muscle during disuse, functional assessment of, e.g., twitch torque requires specialized equipment and may not always be possible in a 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 noninvasive 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 (MHC), at least in the vastus lateralis muscle (39) and possibly also in other muscles. There is a clear distinction between results obtained from twitch 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).

With the use of TMG, it was found that after 35 days of bed rest, there was no change in the Tc of the vastus medialis but an increased Tc in gastrocnemius medialis muscle (34). The authors did, however, report that the TMG amplitude [displacement (Dm)] was increased in both muscles and that for gastrocnemius medialis, the change in Dm was negatively correlated to the change in thickness ($r = -0.70$). The Dm increase in both muscles in the above-mentioned study may indicate a lower muscle resting tension and possibly, decreased viscoelasticity (16).

Whereas 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 the following: *1*) the time course of changes in muscle architecture and TMG parameters during 35 days of bed rest and the following 30 days of 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 yr; SD 2.6; 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 partic-

ipants 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 before the study. All procedures were in accordance with the ethical standards in the 1964 Declaration of Helsinki and its amendments.

Experimental design. The bed-rest study was conducted in the Orthopaedic Hospital of Valdoltra under medical supervision. Participants arrived 1 week before the bed rest and were asked to visit the laboratory on several occasions to become familiar with testing procedures. All baseline data were collected (BDC) 1 day before the start of bed rest. After BDC, participants went through 35 days of 6° head-down tilt bed rest, followed by 30 days of supervised recovery. Subsequent measurements were performed at *days 1–10*, *16*, *28*, and *35* of bed rest (BR1–BR10, BR16, BR28, and BR35, respectively) and *days 1*, *3*, and *30* after completion of bed rest (reambulation; R + 1, R + 3, and R + 30, respectively). During recovery, a fitness professional was available, and all participants received written recovery instructions. Recovery consisted of 12 sessions (3 sessions/ week). Each session lasted ~60 min and consisted of a 10-min warm-up, 5 min active stretching, followed by 20 min strength and balance exercises, 20 min aerobic exercises, and a 5-min cool-down.

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

Ultrasonography. Muscle architecture was determined at rest with B-mode ultrasonography (MyLab 25, 13–4 MHz linear array transducer probe LA523; Esaote Biomedica, Geneva, Italy). Biceps femoris (BF) scans were taken with the participant prone and with a knee angle set at 5° flexion with foam pads. The BF measuring site was half-way between the ischial tuberosity and the posterior 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 millimeters) and pennation angle (Θ; in degrees) were measured using

Table 1. *Anthropometric data of participants*

	BDC	BR35	R + 30	P (η^2)
n	10			
Body height, m	1.78 (SD 6.5)	1.78 (SD 6.5)*	1.78 (SD 6.6)	0.92
Body mass, kg	75.3 (SD 9.3)	72.2 (SD 8.7)†	74.8 (SD 8.2)	<0.001 (0.709)
Fat mass, kg	15.8 (SD 3.6)	15.7 (SD 3.2)	14.4 (SD 2.6)‡	0.003 (0.470)
Body mass index, kg/m^2	23.7 (SD 1.9)	22.7 (SD 1.7)†	23.6 (SD 1.7)	<0.001 (0.700)

Values are means ± SD; *n* = number of participants. BDC, before bed rest; BR35, 35 days bed rest; R + 30, after 30 days recovery. *Body height was measured 12 h after reambulation. †$P < 0.001$ significantly different from BDC. ‡$P < 0.01$.

Matlab (MathWorks, Natick, MA). 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 were used for further analysis.

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 Dm (in millimeters) increased no further. Electrical pulses ranged between 85 and 110 mA at constant 30 V. From two maximal twitch responses, also Tc (in milliseconds) was calculated (Fig. 1) as the time for the amplitude to increase from 10% to 90% of Dm (Fig. 1) (39, 42). Furthermore, the velocity of radial displacement (V_r) was calculated by dividing $0.8 \cdot$ Dm with Tc (37).

Statistics. SPSS software (IBM Ltd., Armonk, NY) was used for all statistical analyses. All data in text and tables are presented as means with SD, whereas in figures, SE was 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 × muscle interaction were found, then 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 × 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 with the BDC value) for each muscle. Pearson regression analysis was used to correlate relative changes during bed rest (BR35 − BDC)/BDC in Tc and Dm to changes in muscle architecture. Statistical significance was accepted at $P \leq 0.05$. The effect size for dependent variables was given as partial η^2.

RESULTS

The variations in muscle structure, as determined by ultrasonography, and muscle contractile function, as measured with TMG, are reported in Fig. 2. Skeletal muscle thickness changed during the study ($P < 0.001$; $\eta^2 = 0.865$; Fig. 2A). Specifically, thickness declined progressively by 4.5% at BR7 ($P = 0.048$) to 15.2% at BR35 ($P < 0.001$) and recovered to BDC thickness at R + 30 ($P = 0.22$). The absence of a time × muscle interaction ($P = 0.50$) indicates that the percent changes in muscle thickness during bed rest and recovery did not differ significantly between muscles.

The time × muscle interaction ($P < 0.001$; $\eta^2 = 0.938$) for Θ indicates that the changes in Θ over time differed among the three muscles. Whereas the time course was qualitatively similar for the three muscles ($P < 0.001$; $\eta^2 = 0.592$; Fig. 2B), post hoc analysis revealed that in the VMO, Θ was first significantly decreased at BR6 (13.6%; $P = 0.033$), whereas in VML and BF, it was already decreased at BR2 (5.5%; $P = 0.037$) and BR3 (7.4%; $P = 0.019$), respectively, interestingly at a smaller decrease due to lower variance. In VMO and VML, Θ had recovered to BDC at R + 30 ($P > 0.05$), whereas in BF, it was already recovered at R + 3 ($P = 0.32$).

Two parameters characterize the TMG signal—Dm and Tc—as well as the ratio between them, the V_r. The muscle × time interaction for Dm ($P < 0.001$; $\eta^2 = 0.186$) indicates that the changes in Dm during the study ($P < 0.001$; $\eta^2 = 0.782$) differed among the three muscles (Fig. 2C). Whereas 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 ($P = 0.013$; $\eta^2 = 0.381$). Dm increased already after BR1, BR4, and BR6 in VMO, VML, and BF, respectively, and had returned to BDC at R + 3 ($P = 0.050$).

The muscle × time interaction for Tc ($P < 0.001$; $\eta^2 = 0.255$) indicates that the changes in Tc during the study ($P < 0.001$; $\eta^2 = 0.397$; Fig. 2D) differed among the three muscles. Post hoc analysis revealed that the Tc of the VMO did not change significantly during bed rest and recovery ($P = 0.35$), whereas the Tc of the VML ($P < 0.001$; $\eta^2 = 0.300$) and BF ($P < 0.001$; $\eta^2 = 0.393$) 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%; $P = 0.043$), being
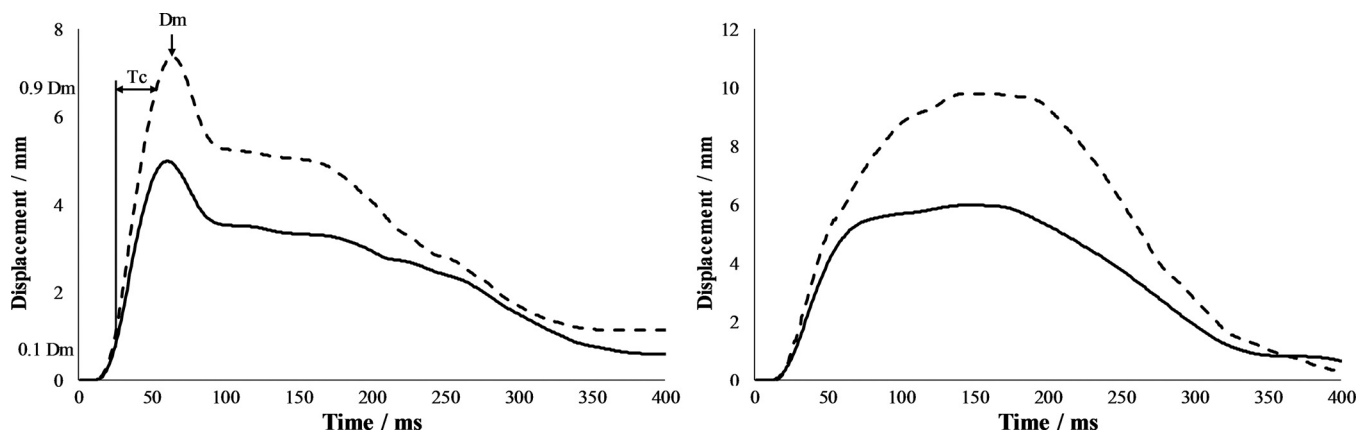

Fig. 1. Typical tensiomyographic response of the vastus medialis obliquus (left) and biceps femoris (right) at baseline (solid lines) and after 35 days of bed rest (dashed lines). Tc, contraction time defined as the time from 10% to 90% of the maximal displacement amplitude (Dm).
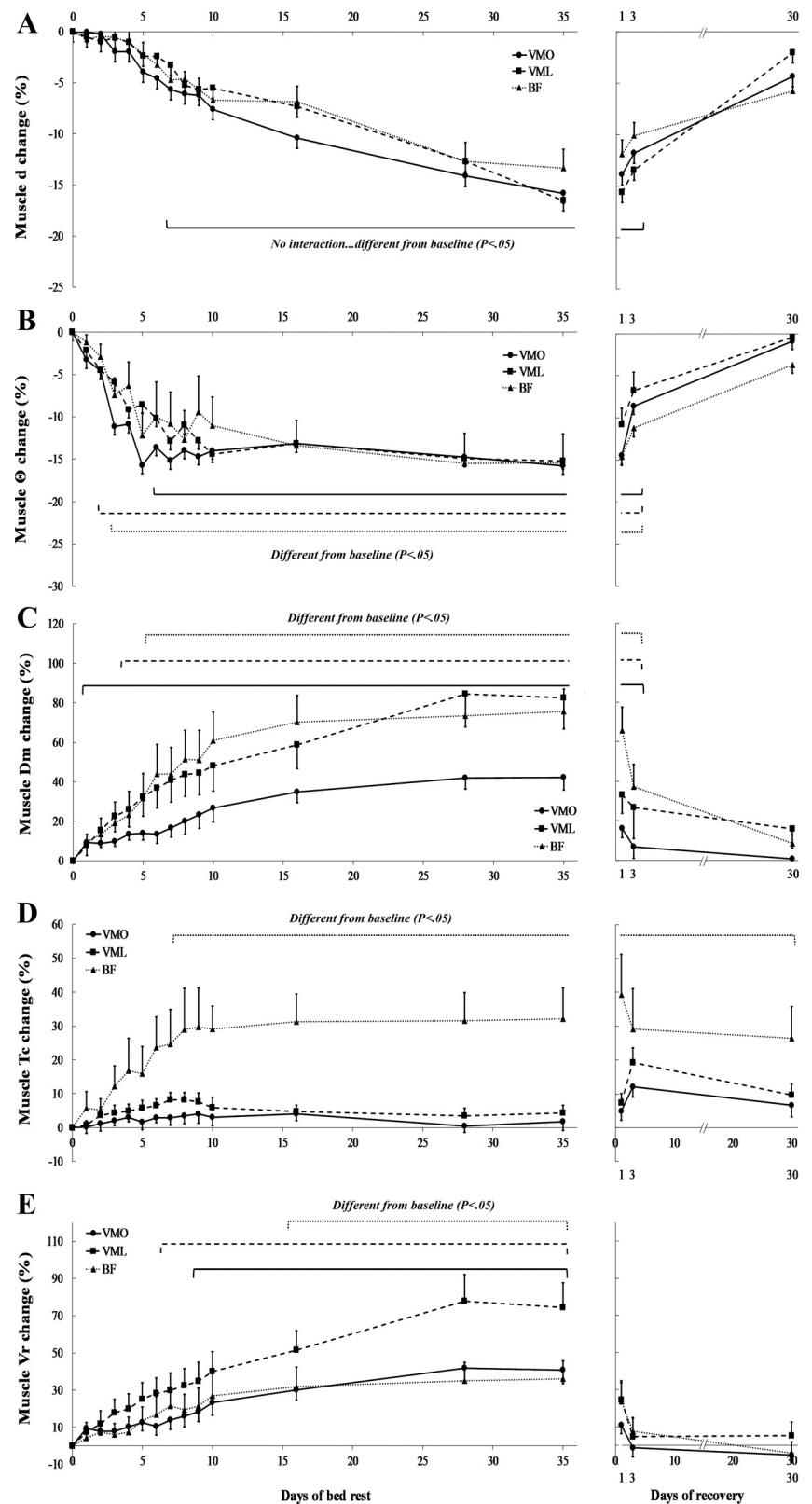
Fig. 2. Changes in (A) thickness (d), (B) pennation angle (Θ), (C) tensiomyographic displacement (Dm), (D) contraction time (Tc), and (E) velocity of radial displacement (V_r) during 35-day bed rest and 30-day recovery in the vastus medialis oblique (VMO), vastus medialis longus (VML), and biceps femoris (BF). Values are means ± SE.

highest at R + 1 (39.3%; $P = 0.013$). BF Tc did not return to the BDC value even at R + 30 (26.4%; $P = 0.041$).

The muscle × time interaction for V_r ($P < 0.001$; $\eta^2 = 0.283$) indicates that the changes in V_r during the study

($P < 0.001$; $\eta^2 = 0.733$; Fig. 2E) differed among the three muscles. We found differences in V_r at BDC ($P = 0.017$), where V_r was slowest in BF compared with VM muscles ($P = 0.014$). Furthermore, post hoc analysis revealed that V_r of the

VMO, VML, and BF increased during bed rest for 40.7% ($P <$ 0.001; $\eta^2 = 0.609$) after BR9, for 74.6% ($P < 0.001$; $\eta^2 = 0.679$) after BR6, and for 36.1% ($P < 0.001$; $\eta^2 = 0.418$) after BR16, respectively. In all muscles, V_r returned to BDC at R + 1.

The contractile parameters measured with TMG and the structural parameters measured with ultrasonography revealed correlations (Fig. 3). Changes in muscle thickness and Dm between BDC and BR35 were negatively correlated. This negative correlation was significant in the BF ($P = 0.001$) but not in the VMO ($P = 0.09$) and VML ($P = 0.06$). There was also a positive correlation between Dm and Θ in VMO ($P = 0.008$) and VML ($P = 0.050$).

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 bed 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. Whereas 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 detect noninvasively and easily 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, although 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 toward 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 h 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 of 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 the decrease in muscle tone, resulting in 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%,
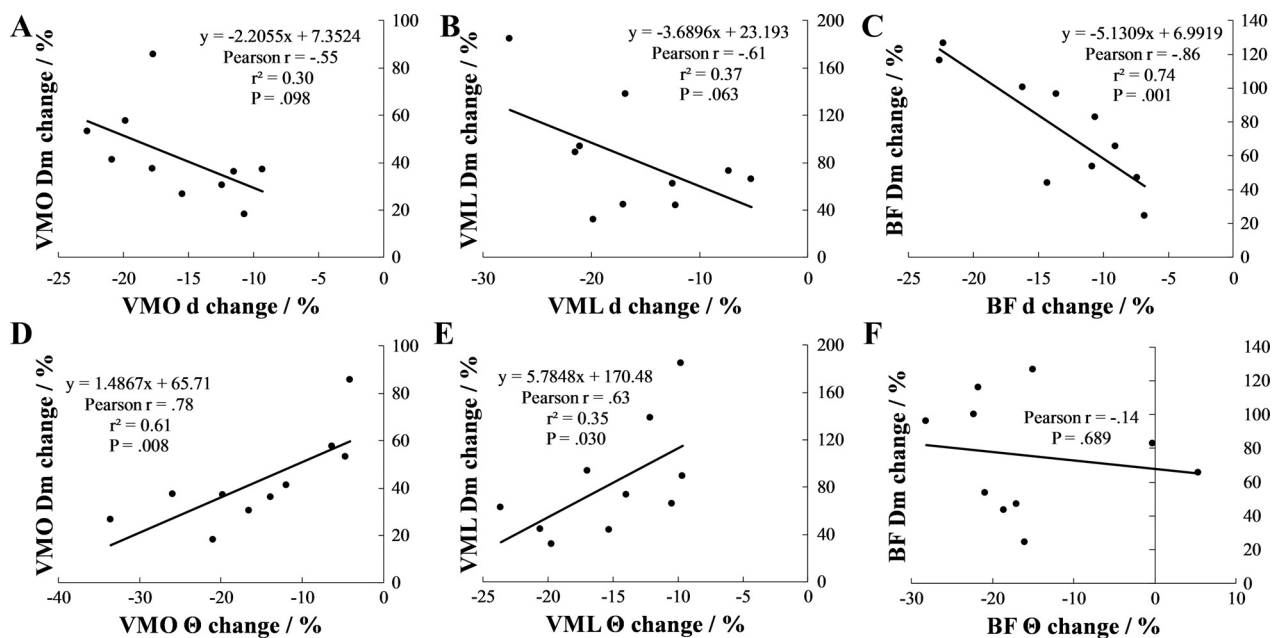

Fig. 3. Pearson correlations between changes in tensiomyographic displacement (Dm) after 35-day bed rest and thickness (d; *A–C*) and pennation angle (Θ; *D–F*) in the vastus medialis obliquus (VMO), vastus medialis longus (VML), and biceps femoris (BF).

in parallel with a 12.3% reduction in plasma volume in just 4 days (19). After the 4th 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).

Furthermore, dry immersion induces an increase in Dm and decrease in muscle-tendon viscoelasticity (10, 23) that is, at least partly, attributable to a similar fluid shift away from the muscles. A decrease in muscle tone, which occurs as early as after 1 day of dry immersion, may further contribute to the increased Dm after 3 days of dry immersion (10) and after 20 days of bed rest (23). Such changes have indeed been observed to translate into higher transversal muscle oscillations during voluntary and electrically evoked contractions (27).

Recent data show that merely a few days (e.g., 5–7 days) of disuse substantially reduce skeletal muscle mass (11, 12), with a slower recovery rate in seniors than in young adults (33, 43). As a consequence, it has been suggested that the accumulation of such short (<10 days), successive periods of bed rest or immobilization during short-term illness or hospitalization may contribute to the loss of muscle mass and metabolic decline observed throughout life (14, 46). Given this slow recovery in the older person and being more prone to hospitalization, it is important to minimize or even prevent any atrophy. Identification of early functional and structural markers of muscle deconditioning may help in the design of adequate interventions to slow such atrophy even before it becomes overt and assess the success of an intervention to prevent atrophy (10). Our data show that Dm may be such a functional marker, a parameter that can be determined with high reproducibility (38, 44).

Bed rest did not induce a significant change in the Tc in the VMO but did induce an increase in the Tc in the VML and BF muscles. That observed increase was much more pronounced for BF, where Tc also did not recover until 30 days after bed rest. Previously, we found a positive correlation between Tc and the MHC-I proportion in vastus lateralis (39), and given that disuse is often associated with a slow MHC-I to fast MHC-IIx transition, the correlation may not apply to disused muscles, where, for instance, a decreased viscoelasticity may have a larger—and opposite—effect than the MHC transition. However, the velocity of V_r increased in all muscles, resulting from increased Dm and the following: *1*) unchanged Tc in VMO, *2*) slightly increased Tc in VML, and *3*) substantially increased Tc in BF. Although V_r should not be paralleled to the contractile velocity of the whole muscle, it is evident that V_r is sensitive to muscle disuse, as well as to assess peripheral fatigue after training (8a) or peripheral arterial disease (18). However, further research is needed for the interpretation of V_r 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 with previously published data, the magnitude of the increase in Tc after 35 days of bed rest was comparable to or even more pronounced than that of sedentary childhood/adolescence or sedentary aging (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 a higher vertical jump (51). The increase in Tc following bed rest in the BF, which was found also in seniors

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

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

Values are means (with SD); n = number of participants.

(42), may thus have significant clinical implications for the quality of life after hospitalization. Therefore, Tc of the BF is a parameter, like Dm, of special interest in assessing the efficacy of therapeutical interventions of people experiencing any kind of disuse, especially in the older population (33, 43).

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 muscles during head-down bed rest and/or are 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, then it is probably worthwhile to assess whether TMG can be used as a 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).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.Š., J.R., S.L., R.P., M.N., and H.D. conceived and designed research; B.Š., S.L., and E.R. performed experiments; B.Š., K.K., J.R., and H.D. analyzed data; B.Š., K.K., J.R., S.L., C.R., E.R., R.P., M.N., and H.D. interpreted results of experiments; B.Š. and H.D. prepared figures; B.Š., J.R.,

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