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# Evaluation of ultrasound data from the MARES Sinusoidal Perturbation Protocol for the analysis of vibration-induced changes in fascicle length and pennation angle as a function of vibration frequency and muscular preload

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## ABSTRACT

**Background:** Vibration exposure has proven to be a useful training method and examination method in a variety of contexts. Nevertheless, not much is known about the mechanical properties of muscle tendon unit during vibration exposure. Therefore, the main aim of this study was to get an insight into vibration-induced changes in fascicle length and pennation angle at different frequencies and levels of contraction.

**Methods:** 23 subjects performed the sinusoidal perturbation protocol before and after 60 days of bed rest. Ultrasound videos of gastrocnemius medialis were recorded during vibration exposure at 10 different frequencies (4 – 16 Hz) and 4 levels of contraction (0, 25, 50 and 75% MVC). Excursion of fascicle length (FL) and pennation angle (PEN) was evaluated using an ultrasound tracking software.

**Results:** Excursion of FL and PEN was significantly smaller when muscle was contracted. For the range from 7 to 10 Hz there was a significant increase in excursion for pre-tensioned muscle, especially for FL. After bed rest, excursion was significantly greater at 9 Hz and 10 Hz vibration for FL and at 8, 9 and 10 Hz for PEN, respectively.

**Conclusion:** Fascicle excursion decreases as the muscle contracts. However, no further correlation between the level of contraction and the decrease in excursion could be found. The main finding of this study was a significant resonance effect at 7-10 Hz vibration and contracted muscle. Resonance was even more evident after bed rest.

**Keywords:** vibration, fascicle architecture, isometric contraction

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## ABBREVIATIONS

WBV:	whole body vibration
FL:	fascicle length
PEN:	pennation angle
MT:	muscle thickness
MVC:	maximum voluntary contraction
PEH:	perievent histogram
MARES:	muscle atrophy and research system
GM:	gastrocnemius medialis
MTU:	muscle-tendon unit
BDC:	baseline data collection
R5:	recovery day 5

## BACKGROUND

Vibration exposure, mostly applied as whole-body vibration (WBV), has been used more and more frequently in recent years in sports, health, research, preventive medicine and rehabilitation. A variety of promising applications has already

been demonstrated. For example, vibration was used to evoke stretch reflex activities (Lambertz et al. 2002). Controlled sinusoidal length perturbation of low amplitude and different frequencies can be considered as a stretch reflex, mediated via a spinal reflex pathway. Cochrane et al. (2009) analyzed contractile tissue displacement and the temporal association of EMG activity in calf muscle by applying a 6 Hz vibration stimulus to their subjects. Beside the fact that vibration exposure has proven to be a useful method to examine the muscle tendon unit (MTU) and to study its mechanical properties, vibration is a sufficient training method in various contexts. Enhancement of average velocity, force and power as well as an increased vertical jump height resulted immediately after vibration treatment (Bosco et al. 1999, Cochrane and Stannard 2005). WBV has shown to be effective in improving balancing abilities in elderly women, providing WBV to potentially reduce risks of fall (Cheung et al. 2007). Long term vibration treatment to patients with spinal diseases resulted in a significant reduction

in pain sensation and pain-related disability (Rittweger et al. 2002) and improved leg extension power, timed-up-and-go test and chair-rising time in elderly people (Bruyere et al. 2005). Even a one-off session of WBV suggested a positive influence on the postural control and mobility in multiple sclerosis patients (Schuhfried et al. 2005). Due to these results it is important to have a better understanding of the impact of vibration on the human body, the MTU and the muscle itself.

Vibration is a periodic alteration of force, acceleration and displacement over time. Energy is transferred from an actuator (i.e. the vibration device) to a resonator (i.e. the human body, or parts of it) during vibration exposure (Rittweger 2010). When vibration is induced to the human body, it does not simply follow the trajectory imposed by the actuator like a rigid body. Instead, the response of the MTU to vibration is complex due to its spring-damping characteristics and divergent mechanical properties of muscle and tendon and requires further investigations. Therefore, ultrasound imaging combined with automated fascicle tracking has proven to be a robust, reliable and time efficient method to capture in vivo muscle fascicle architecture during dynamic activities (Cronin et al. 2011).

In the body, periodic stretching and shortening cycles of the muscle-tendon complex occur during vibration. This is accompanied by periodic changes in fascicle length (FL) and pennation angle (PEN). However, little is known about the extent to which stretching or shortening depends on muscular preload and frequency. Due to the visco-elastic properties of the muscle, both factors should influence the stress-strain behavior in a speed-dependent manner.

In the present study, sinusoidal perturbation was used to determine stretching and shortening of fascicles in human gastrocnemius muscle. Therefore, the primary aim of this evaluation is to investigate the extent of FL and PEN excursion in gastrocnemius medialis (GM) during vibration at different frequencies and contraction levels. In addition, the influence of long-term bed rest on FL and PEN excursion is evaluated as well as the change in static muscle architecture at different levels of contraction.

## METHODS

### STUDY DESIGN

**AGBRESA.** The evaluated data were recorded during AGBRESA (artificial gravity bed rest study), conducted by the German Aerospace Center in cooperation with the European Space Agency (ESA) and the US space agency (NASA). AGBRESA was the first long-term bed rest study to investigate the use of artificial gravity as a possible countermeasure of preventing the negative effects of weightlessness on the human body like bone and muscle atrophy. During the three-month study, the participants were therefore 'rotated' each day while lying in the DLR short-arm centrifuge. The subjects spend 60 days in bed, angled

downwards towards the head end by six degrees to achieve the displacement of body fluids experienced by astronauts in a microgravity environment. All experiments, meals and leisure pursuits were taken place lying down during the bed-rest phase. Additionally, participants movements were restricted to reduce strain on muscles, tendons and the skeletal system (Burtscheidt et al. 2019).

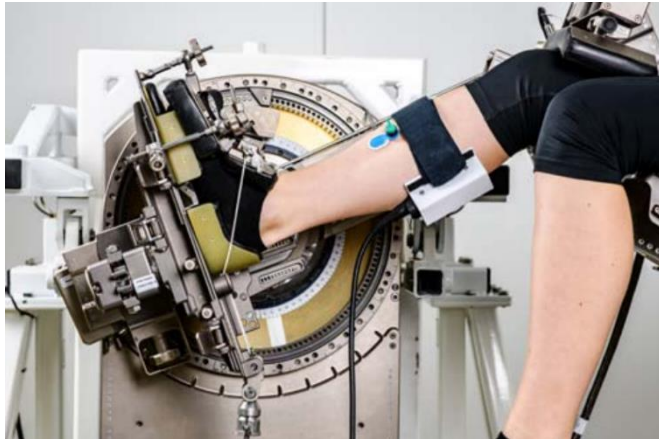
**PARTICIPANTS.** 15 males and 8 females with an average (mean,  $\pm$  SD) age  $33.3 \pm 9.0$  years, height  $174.6 \pm 8.4$  cm and body weight  $74.2 \pm 9.8$  kg participated in two campaigns of this long-term bed rest study. Subjects were randomly grouped into following three countermeasure groups:  
 continuous artificial gravity (cAG group): 30 minutes of continuous centrifuge drive per day  
 intermittent artificial gravity (iAG group): five minutes of centrifuge drive for six times per day  
 no artificial gravity (control group): no centrifuge drive

**SARCOLAB BR.** The Sarcolab BR protocol is a set of different measurements with the MARES device, performed pre- and post-bed rest. MARES (muscle atrophy and research system) initially was used on the International Space Station to investigate the effects of weightlessness to the musculoskeletal system. Several exercises and measurements can be performed by the MARES device, which can be set up individually for each subject. The aim of the Sarcolab BR experiment was, inter alia, to characterize reflex excitability of muscles, investigate elastic and mechanical properties of muscle-tendon-complex and to assess muscle architectural features.

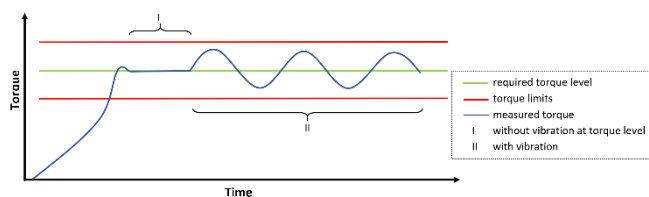
**SINUSOIDAL PERTURBATION PROTOCOL.** In this report, the Sinusoidal Perturbation (SP) measurement, which was one experiment of the Sarcolab BR protocol, is to be evaluated. During SP protocol subjects were seated into the MARES with the chair and pantograph set to obtain a  $60^\circ$  knee angle from full extension and the ankle was securely fixed to the ankle adapter at  $90^\circ$  ankle joint angle (Fig. 1). An ultrasound probe was positioned at the medial head of the gastrocnemius along its mid-sagittal axis fixed by a custom-made mount and elastic strap. Several electromyographic surface electrodes were attached to the leg, however, EMG data are not considered further in this evaluation.

The SP measurement includes vibration of ten frequencies (4, 5, 6, 7, 8, 9, 10, 12, 14 and 16 Hz) each at four levels of muscular preload (25, 50, 0 and 75% MVC in this order). MVC (maximum voluntary contraction) was previously measured in Sarcolab BR protocol. 50 cycles of sinusoidal oscillation were induced to the foot by MARES, starting when the subject reached and maintained the required contraction level (isometric plantar flexion torque), except for the 0% MVC measurements, where the muscle was relaxed during vibration (Fig. 2). Amplitude of oscillation was  $3^\circ$  total ( $-1.5^\circ$  to  $+1.5^\circ$ ) around the neutral ankle position. A screen visualized the subject's applied torque including a target line

and boundary lines to maintain the contraction level within these limits over the entire vibration sequence (Fig. 2). Between contractions there were rest periods of 10 s for 25% and 50% MVC, 5 s for 0% MVC and 15 s for 75% MVC independent of the frequency. Measurements of the combinations 50% MVC at 6 Hz and 7 Hz and 75% MVC at 12 Hz were performed and recorded twice to verify the reliability of the measurement and evaluation method.



**Figure 1. MARES setup. Knee angle at 60°, ankle joint angle at 90°, ultrasound probe fixed in mount and attached to lower leg.**



**Figure 2. Test procedure. Torque must be set to a specified level and maintained until the vibration stops.**

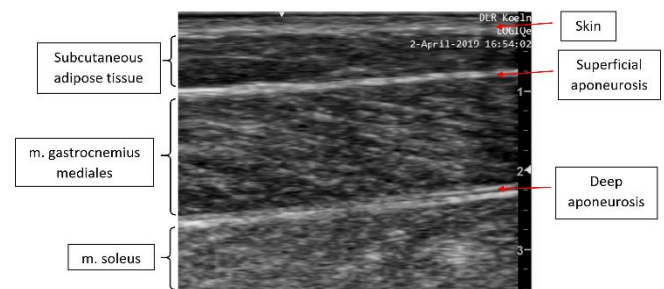
## DATA ACQUISITION

B-mode ultrasound data was recorded at a sampling rate of 139 frames/s, using a linear transducer with an ultrasound frequency of 9 MHz. Scanning depth was adjusted between 30 mm and 50 mm so that the medial gastrocnemius muscle fascicles and the adjacent aponeuroses were visible clearly in the ultrasound video (Fig. 3).

## DATA ANALYSIS

**UltraTrack.** For this evaluation two data sets of each participant were analyzed. One recorded before bed rest (baseline data collection, BDC) and the other one five days after bed rest phase (recovery day 5, R5). Three subjects had to terminate the study and one ultrasound data set was unusable for subsequent analysis, giving a total of 42 data sets with 40 videos each. The ultrasound sequences were

evaluated by using UltraTrack, an algorithm for semi-automated tracking of muscle fascicle length in dynamic ultrasound recordings. The ultrasound data was converted to AVI video files to be readable with UltraTrack. After loading the input file, the first ultrasound frame is displayed. For calculating the correct scale of the image (pixels/mm), any borders above and below the image of the first frame were removed and the scanning depth was input. The region of interest (ROI) was defined by outlining the gastrocnemius medialis including the adjacent aponeuroses (Fig. 4). Two straight lines were defined in the ROI: One as the actual muscle fascicle, following the structure of the intramuscular connective tissue between the superficial and the deep aponeurosis, and one representing the anterior gastrocnemius aponeurosis, which is needed to calculate the pennation angle. By running the tracking algorithm, the defined fascicles are tracked throughout the image sequence (Fig. 4). The tracking algorithm is based on the affine optic flow model, which steps through the image sequence one frame at a time, computing the optic flow between consecutive images and applying the affine transformation to calculate the new position and length of the fascicle. Because this is an iterative process, any tracking errors in individual frames may accumulate over time and be compounded, causing a ‘low frequency drift’ (Farris and Lichtwark 2016). Once the algorithm has finished, the resulting tables including time, fascicle length and fascicle angle to the horizontal axis were saved as TXT data for further evaluation as well as a MAT file containing the tracking data of the first frame, which was saved to calculate the correct pennation angle.



**Figure 3. Ultrasound image of calf muscle (gastrocnemius medialis).**

**R-SCRIPTS.** The evaluation of the tracking data was performed using RStudio, the integrated development environment for R, in its version 1.3.959 ([www.rstudio.com](http://www.rstudio.com)).

**STATIC DATA ANALYSIS.** To analyze the static fascicle architecture (absolute values of FL, PEN and MT) at different levels of muscular preload (0, 25, 50 and 75% MVC), the fascicle length and pennation angle values just before the start of the vibration are needed, where the required torque level is reached (Fig. 2, phase I). The algorithm reads out the vibration start frames and calculates

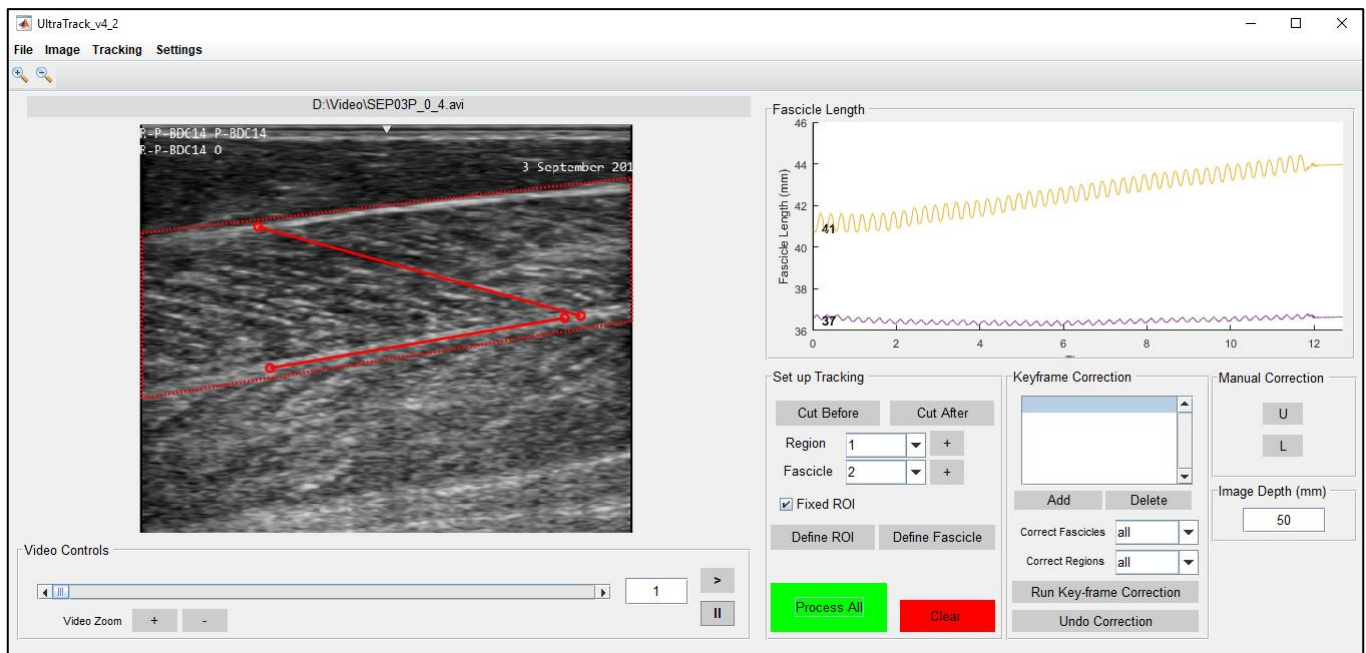


Figure 4. UltraTrack tracking software: ROI, fascicle and deep aponeurosis defined in the first frame (left), fascicle length plotted after processing (right).

the average FL and PEN values of these frames for every subject and every level of muscular preload. The PEN is calculated by either adding or subtracting the aponeurosis angle from the fascicle angle, depending on the position of the aponeurosis. Therefore, the algorithm checks the position of the two aponeurosis endpoints. With the resulting FL and PEN data the fascicle shortening and angular enlargement by increasing muscle tension can be shown (Fig. 5). Muscle thickness was calculated as  $mt = fl * \sin(pen)$  by the values of fascicle length and pennation angle.

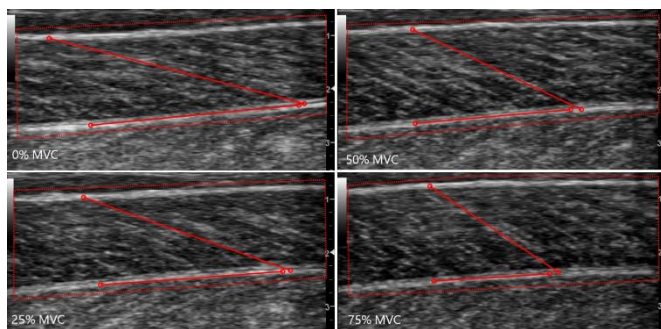


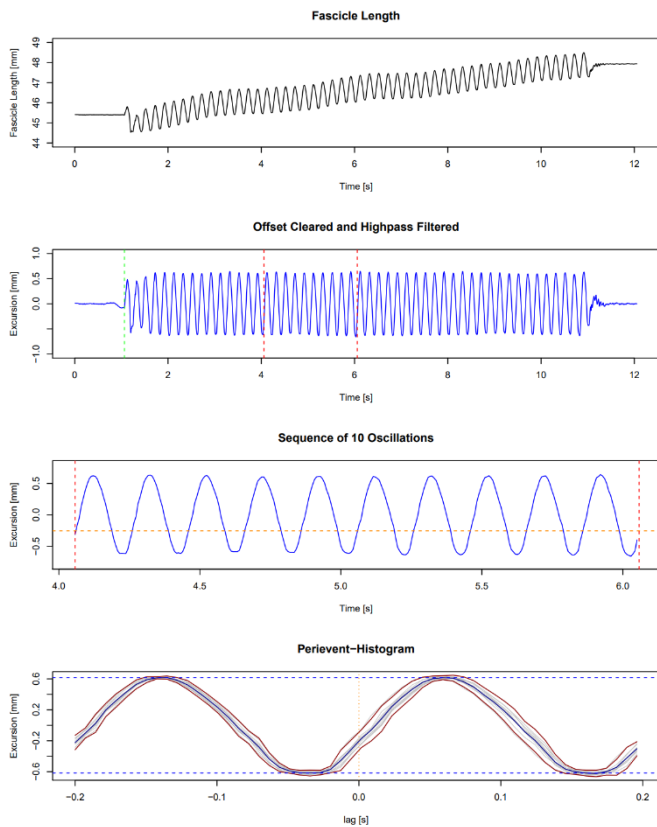
Figure 5. A typical change in muscle architecture by increasing contraction levels.

**VIBRATION DATA ANALYSIS.** To analyze the response of fascicles to oscillating excitations, the mean FL and PEN excursion during perturbation is required. Perievent histograms (PEH) were used to determine the excursion values from the tracking data. Therefore, the offset value was set to zero by subtracting the initial value from all FL or PEN values of the sequence. As mentioned previously, low frequency drifts may appear in some data streams, which were

straightened out using a high pass filter. The PEH algorithm then superimposed 10 oscillations of the vibration sequence. For this reason, a vibration sequence with the length of 10 oscillation periods was extracted, omitting the first 15 oscillations to avoid integrating any transient effects. The single oscillations of this sequence were then overlaid. For this purpose, whenever exceeding a prespecified threshold value, the sequence was interrupted at the intersection and the next oscillation was overlapped until the threshold value was passed again and so on until all oscillations were overlaid (Fig. 6). Due to smaller peaks and irregularities occurring in the oscillations sequence, a dead time was implemented to prevent interrupting the stream within one oscillation period. After calculating the average oscillation curve, the maximum and minimum values were determined, corresponding the mean excursion values. From each PEH the following information was extracted: the positive, negative and total excursion as well as the relative values of positive, negative and total excursion correlated to the initial value of FL or PEN.

**TEST-RETEST RELIABILITY.** Reliability was verified by comparing the results of the repeated measurements. Therefore, the mean coefficient of variation (CV) was determined by first calculating the coefficient for all individual pairs of values and then forming the average value from these coefficients. Coefficient of variation is calculated by  $sd/mean * 100$  and is given in percent. The mean coefficient of variation was calculated for relative FL and PEN excursion and for the static values of FL and PEN.





**Figure 6. Development process of perievent histograms.** 1) Fascicle length data plot. 2) Offset (initial value) is set to zero and high pass filter was applied, start of perturbation (green dotted line), start and end of sequence to be analyzed (red dotted line). 3) Sequence of 10 oscillations and threshold value (orange dotted line). 4) Perievent Histogram: Overlapping oscillations when passing the threshold value, positive and negative excursion (blue dotted lines).

**STATISTICAL ANALYSIS.** All statistical analyses were performed using a custom-made R-script. Linear mixed effects (LME) models with frequency (only for vibration excursion analysis), MVC level, group and time as fixed effects and subjects as random effects were created to assess the main and interaction effects on the dependent variables (static FL, PEN and MT values and vibration excursion values of FL and PEN, respectively). The level of statistical significance was set to  $\alpha = 0.05$  and  $\beta$  was set to 0.45. Due to a heteroscedastic distribution of the residuals of the excursion values a boxcox transformation was applied. Linear mixed-effects models with all possible interaction terms were constructed and stepwise simplified where  $P > \beta$ . Significant effects observed in the ANOVA were followed up by a-priori defined treatment contrasts. Reference levels for contrasts were 4 Hz, 0% MVC, control group and baseline data (BDC) for excursion analysis and 0% MVC, control group and baseline data for static fascicle analysis.

## RESULTS

As already mentioned above, the post bed rest data sets of four subjects are missing. In order to keep the evaluation as consistent as possible, the corresponding BDC data of these

subjects were excluded in all evaluations where BDC and R5 data were compared. However, all BDC data sets were used in evaluations that contained only pre bed rest data.

## TEST-RETEST RELIABILITY

The results of the reliability are shown in Table 1. Mean coefficient of variation was 13.6% for FL excursion, 18.2% for PEN excursion and about 5% for fascicle architecture data. Considering all possible limitations (ultrasound quality, tracking errors, inaccuracies in the identification of the fascicles), the results can be considered acceptable and the measurement and evaluation method has proven to be reliable.

Table 1. Mean Coefficient of Variation				
	rel. exc. FL	rel. exc. PEN	static FL	static PEN
coeff. of variation [%]	13.6	18.2	4.5	5.7

## STATIC FASCICLE ARCHITECTURE

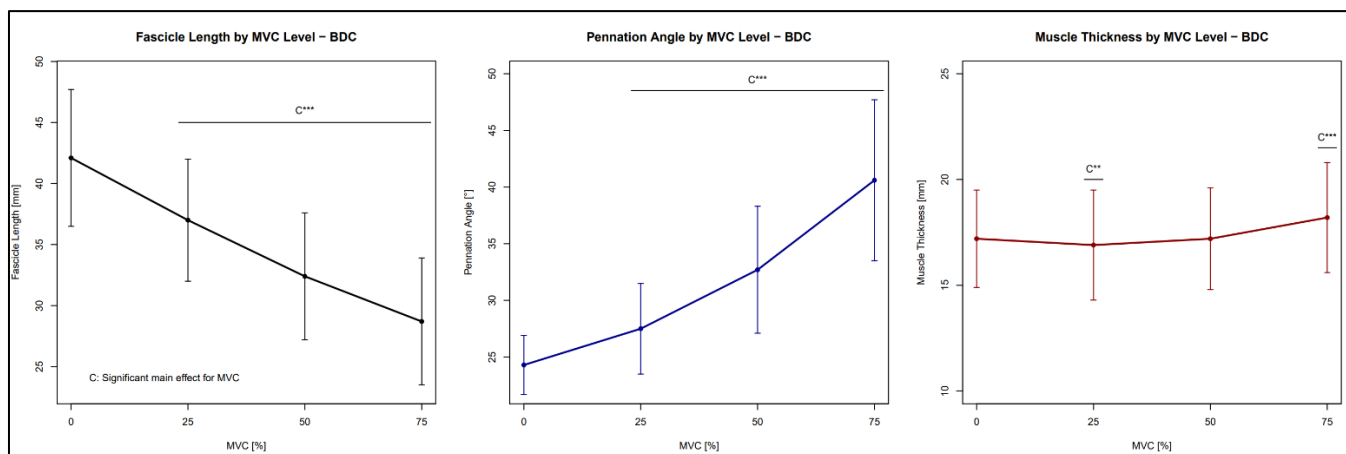
**BDC DATA.** First, only the baseline data of the fascicle architecture by different contraction levels shall be considered. As the main effects for group were not statistically significant ( $P = 0.50$  for FL,  $P = 0.13$  for PEN,  $P = 0.43$  for MT), the baseline data of the three countermeasure groups were lumped together. Fascicle shortening and pennation angle enlargement occurred by increasing levels of contraction (Table 2, Fig. 7). FL was significantly smaller for 25% MVC ( $37.0 \pm 5.0$  mm,  $P < 0.001$ ), 50% MVC ( $32.4 \pm 5.2$  mm,  $P < 0.001$ ) and 75% MVC ( $28.7 \pm 5.2$  mm,  $P < 0.001$ ) compared to 0% MVC ( $42.1 \pm 5.6$  mm). PEN showed statistically significant greater values for 25% MVC ( $27.5 \pm 4.0^\circ$ ,  $P < 0.05$ ), 50% MVC ( $32.7 \pm 5.6^\circ$ ,  $P < 0.001$ ) and 75% MVC ( $40.6 \pm 7.1^\circ$ ,  $P < 0.001$ ) in comparison with 0% MVC ( $24.3 \pm 2.6^\circ$ ). In contrast, there were only minor changes in muscle thickness (Table 2, Fig. 7). MT was statistically significant smaller for 25% MVC ( $16.9 \pm 2.6$  mm,  $P < 0.01$ )

Table 2. Mean (SD) FL, PEN and MT by MVC levels				
	0% MVC	25% MVC	50% MVC	75% MVC
FL [mm]	42.1 (5.6)	37 (5.0)***	32.4 (5.2)***	28.7 (5.2)***
PEN [°]	24.3 (2.6)	27.5 (4.0)*	32.7 (5.6)***	40.6 (7.1)***
MT [mm]	17.2 (2.3)	16.9 (2.6)**	17.2 (2.4)	18.2 (2.6)***

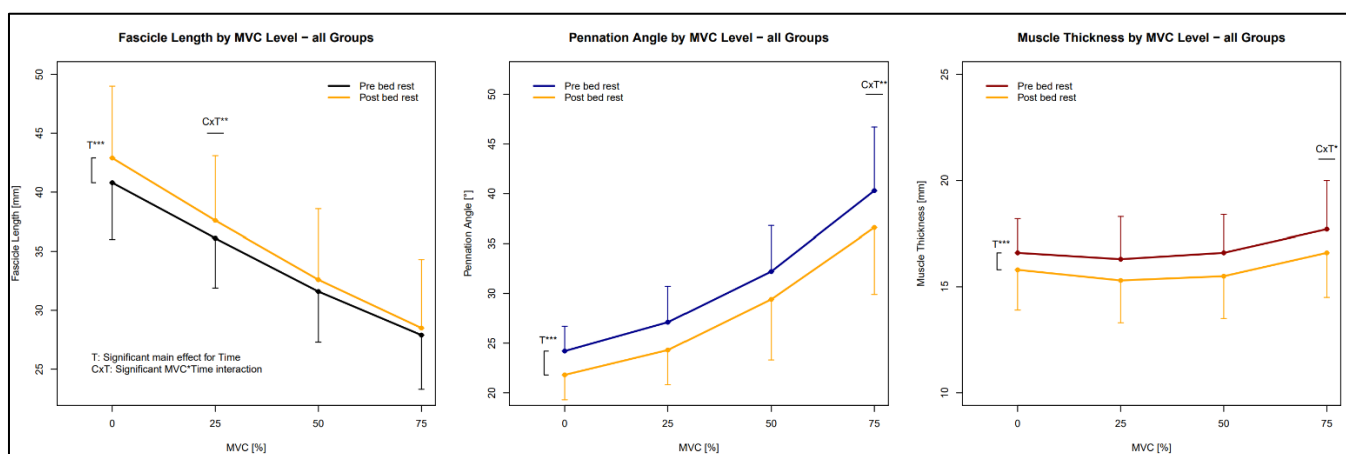
\*\*\* P < 0.001, \*\* P < 0.01, \* P < 0.05

Table 3. Mean (SD) FL decrease and PEN increase between MVC levels			
	0-25% MVC	25-50% MVC	50-75% MVC
FL decrease [mm]	5.2 (2.0)	4.5 (2.2)	4.1 (2.2)
PEN increase [°]	3.2 (2.1)	5.2 (3.6)	8.1 (4.2)***

\*\*\* P < 0.001



**Figure 7. Mean (SD) baseline fascicle length, pennation angle and muscle thickness by levels of contraction. C denotes significant main effect for MVC with 0% MVC as reference level of contrast (\*\**P* < 0.001, \*\* *P* < 0.01).**



**Figure 8. Mean (SD) fascicle length, pennation angle and muscle thickness pre and post bed rest. T denotes significant main effect for time with BDC as reference level of contrast. CxT denotes significant MVC × time interaction effect with BDC × 0%MVC as reference level of contrast. (\*\**P* < 0.001, \*\* *P* < 0.01, \* *P* < 0.05).**

and significantly greater for 75% MVC ( $18.2 \pm 2.9$  mm, *P* < 0.001) compared to the measurements without contraction ( $17.2 \pm 2.3$  mm).

Considering the mean differences between contraction levels (Table 3), there are no statistically significant differences between FL shortenings. Statistically significant differences occurred in PEN enlargements, having the greatest average PEN increase for 50-75% MVC level ( $8.1 \pm 4.2$  mm, *P* < 0.001).

**R5 DATA.** The results of the fascicle architecture before and after bed rest including all statistically significant main and interaction effects are shown in Fig. 8 and 9. (appendix). There were statistically significant main effects for time in FL, PEN and MT data (Fig. 8). Compared to baseline data, overall values increased for FL ( $+1.09 \pm 0.56$  mm) and decreased for PEN ( $-2.88 \pm 0.7$  °) and MT ( $-1.03 \pm 0.22$  mm) after bed rest. The group × time interaction effect was significant for FL in iAG group (Fig. 9.1-3 in appendix), having greater values at R5 ( $+1.78 \pm 0.77$  mm) than control group. The same tendency emerged for cAG-group ( $+1.35 \pm 0.79$  mm, *P* = 0.09). Although the calculated MT was smaller in all groups

after bed rest than before, there were statistically significant group × time interaction effects for MT showing that MT after bed rest was greater in AG groups than in control group ( $+0.91 \pm 0.31$  mm in cAG group,  $+1.03 \pm 0.30$  mm in iAG group).

### FASCICLE EXCURSION DURING VIBRATION

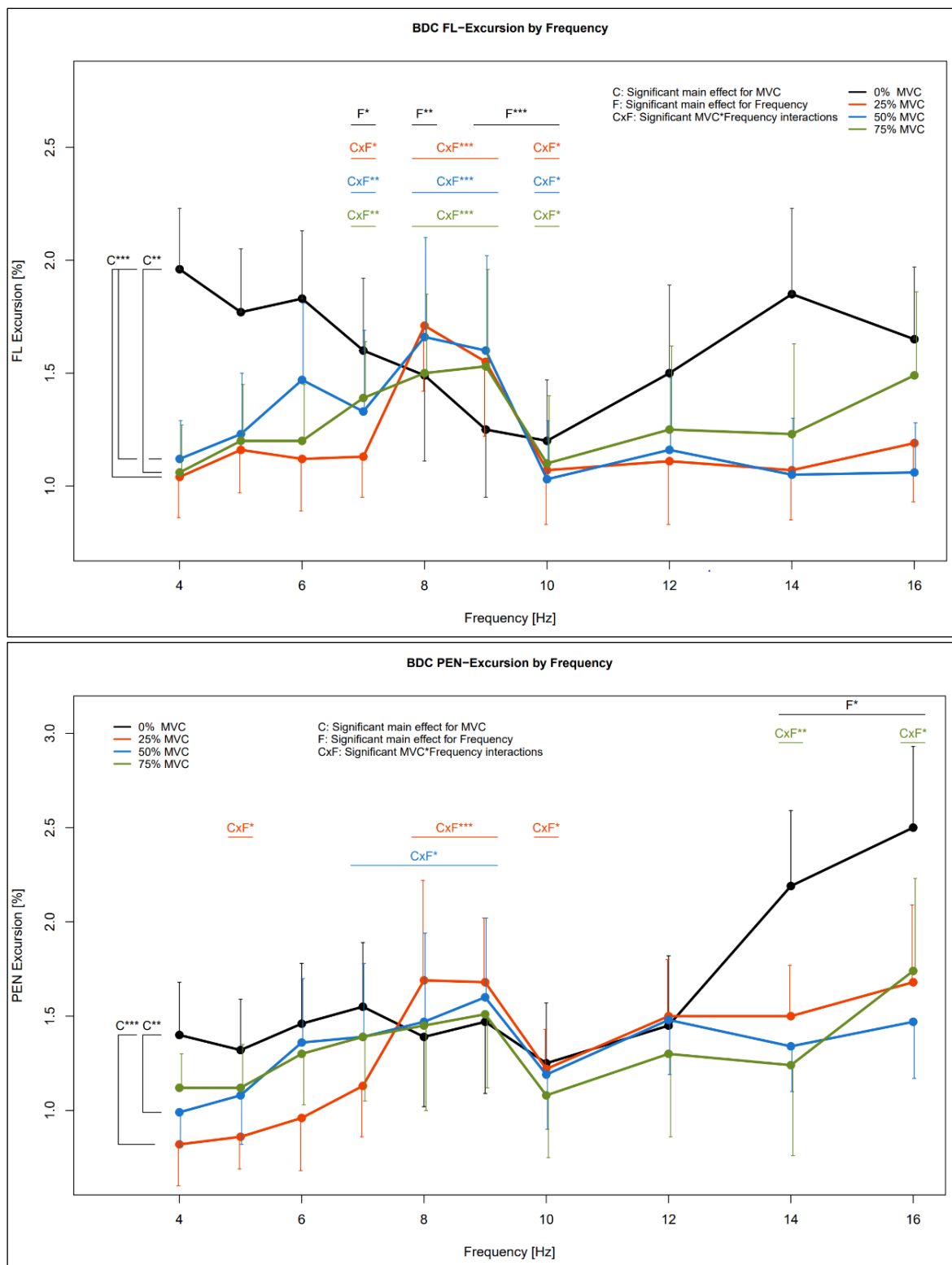
For the analysis of the excursion data only the relative total excursion is considered in the following, which corresponds to the difference between maximum and minimum of FL or PEN with respect to the initial value before being excited by vibration.

### PRE BED REST DATA

First, the influence of contraction and frequency on the extent of fascicle excursion across all groups is considered. The main effects for group were not statistically significant (*P* = 0.38 for FL and *P* = 0.45 for PEN). Therefore, the baseline data of all groups were lumped together for this approach (Fig. 10).

**FASCICLE LENGTH.** There was a significant main effect for contraction in all MVC levels. FL excursion was significantly smaller for 25%, 50% and 75% MVC compared to the 0% MVC measurements. Significant main effects for frequency occurred at 7, 8, 9 and 10 Hz, where the excursion is generally

smaller than for 4 Hz vibration. The same frequencies showed statistically significant MVC × Frequency interaction effects at 25%, 50% and 75% MVC levels, but in contrast, the interaction of these MVC levels with 7 to 10 Hz frequencies resulted in a significant increase in excursion (Fig. 10).



**Figure 10. Mean (95% Confidence Level) relative FL and PEN excursion by MVC and frequency.** F and C denote significant main effects for frequency and MVC, respectively. CxF denotes significant MVC × frequency interactions, with 0% MVC × 4 Hz as reference level of contrast. Color represents the corresponding MVC level. (\*\*\*) P < 0.001, (\*\*) P < 0.01, (\*) P < 0.05).

**PENNATION ANGLE.** The statistical analysis of PEN excursion data showed significant main effects for contraction and frequency. Overall PEN excursion for 25% and 50% MVC levels was substantially smaller compared to relaxed muscle, but not for 75% MVC. PEN excursion was significantly greater at 14 and 16 Hz than at 4 Hz vibration frequency. Significant MVC × Frequency interaction effects appeared for 25% MVC at 5, 8, 9 and 10 Hz and for 50 % MVC at 7, 8 and 9 Hz, resulting in greater excursion values when compared to 4 Hz vibration. However, interaction of 75% MVC with 14 and 16 Hz showed statistically significant smaller excursion (Fig. 10).

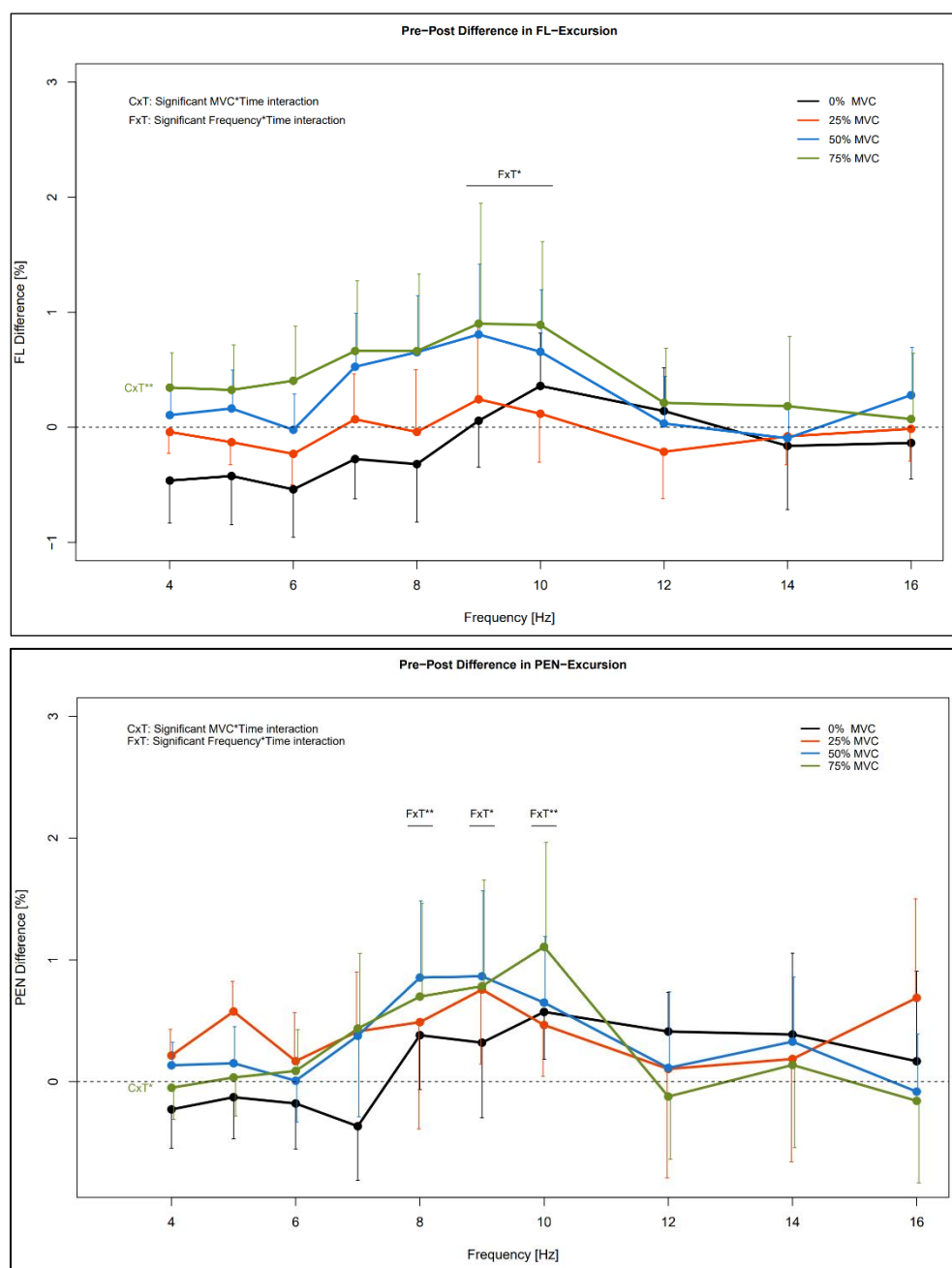
**POST BED REST DATA**

For a better visualization of time effects, the mean (and 95% CL) difference between post- and pre- bed rest is plotted in

the following diagrams including all statistically significant interaction effects (Fig. 11, 12 in appendix).

Significant frequency × time interaction effects resulted for FL and PEN data. After bed rest, excursion of FL was greater at 9 and 10 Hz and excursion of PEN at 8, 9 and 10 Hz. The effect of MVC × time interaction was statistically significant for 75% MVC after bed rest, where excursion increased for both FL and PEN excursion data (Fig. 11).

In iAG-group, the time × group interaction showed a significant decrease of FL excursion. However, MVC × group interaction was significant in this group, showing smaller values for excursion at 50% MVC compared to 0% MVC. In cAG-group MVC × time × group interaction effect was statistically significant for 50% MVC toward greater excursion values (Fig. 12.1 in appendix).



**Figure 11. Mean (95% CL) excursion difference between post and pre bed rest.** CxT denotes significant MVC × time interactions with 0% MVC × BDC as reference level of contrast. Color represents the corresponding MVC level. FxT denotes significant interaction effects of frequency × time, with 4Hz × BDC being level of contrast. (\*\* P < 0.01, \* P < 0.05).



## DISCUSSION

**STATIC FASCICLE ARCHITECTURE.** One part of this study was to investigate muscle fascicle architecture change in human GM during submaximal isometric contractions. During isometric muscle contraction it is to expect that fascicles shorten and pennation angle enlarges.

In the present study, the fascicle length showed almost linear decreases between MVC levels. This leads to the assumption that the extent of muscle fascicle length decrease is linear to the applied contraction intensity (Narici et al. 1996). In muscle thickness only marginal changes occurred between different contraction levels. In contrast, the pennation angle increase was about 1.6 times greater from one contraction level to the next, leading to suggest a geometric explanation, as the muscle system can be modelled as a two-dimensional parallelogram (Huijing and Woittiez 1984, Maganaris et al. 1998). Assuming that the aponeuroses behave as rigid bodies and run parallel to each other and that muscle fibers run straight between the aponeuroses; it is concluded that the inter-aponeuroses distance (muscle thickness) remains constant (Maganaris et al. 1998). If the FL is shortened evenly in such a model (a certain increase in contraction causes a consistent decrease in FL), then the PEN increases exponentially until reaching 90°.

In this study, average initial FL of GM was  $42.1 \pm 5.6$  mm and initial PEN was  $24.3 \pm 2.6^\circ$ . Maganaris et al. (1998) previously reported similar results for the initial values, namely  $45.0 \pm 2.3$  mm for FL and  $22.3 \pm 2.0^\circ$  for PEN. Héroux et al. (2016) reported  $52.2 \pm 8.2$  mm initial FL and  $16.6 \pm 2.3^\circ$  initial PEN and Narici et al. (1996) described a 50.8 mm initial FL and  $15.5^\circ$  PEN but with an ankle angle of  $110^\circ$ .

During contraction to 25% MVC, fascicles shortened by  $12.2 \pm 4.1\%$  in this study. Similar results have been shown by Héroux et al. (2016), who reported a GM fascicle decrease by 8-12% and by Narici et al. (1996), who found a decrease by 8-10%.

In the current study, PEN increased by  $3.2 \pm 2.1^\circ$  between 0% and 25% MVC levels. A comparable  $4^\circ$  PEN increase was observed by Narici et al. (1996) with the ankle at  $110^\circ$ . PEN increase determined by Héroux et al. (2016) was  $1.4^\circ$  and  $4-5^\circ$  by Maganaris et al. (1998).

Thickness of GM at different levels of contraction calculated in this evaluation resulted in mean values between 16.9 mm and 18.2 mm. Similar outcomes were reported by Maganaris et al. (1998) and Narici et al. (1996), who found values at about 17 mm and 16.5 to 21.1 mm, respectively. However, both reported no significant differences in distance between aponeuroses during graduated isometric force and from rest to MVC, respectively.

There is a close agreement in the values of fascicle architecture between the results of the present study and those of previous studies. Nevertheless, it should be remembered that there is a certain degree of pressure on the soft tissue applied by the ultrasound probe, which can have

an influence on the muscle architecture, especially when examining muscles at rest. Although in this study only the deep pennation angle was determined, it should be mentioned that Bolsterlee et al. (2015) reported an underestimation of superficial pennation angles by approximately  $10^\circ$  due to the pressure of the ultrasound probe. Furthermore, there were differences in the above-mentioned studies with regard to position (sitting, prone), knee angle and gender compositions of subjects.

Comparing the fascicle architecture pre and post bed rest, there are divergent trends for fascicle length, pennation angle and muscle thickness. After bed rest PEN and MT values were smaller in all groups whereas FL showed greater values in iAG-group and a tendency toward greater FL in cAG-group. During bed rest, it is reported that muscle atrophy occurs, especially in anti-gravity muscles of lower limb and the back (Pavy-Le Traon et al. 2007). Several previous bed rest studies reported a significant decrease in muscle volume, muscle thickness and cross-sectional area (Abe et al. 1997, Alkner and Tesch 2004, Rittweger et al. 2005). Muscle atrophy is expected to be accompanied by a decline in pennation angle (Boer et al. 2008, Šimunič et al. 2019). Boer et al. (2008) reported a 14.3% decrease in PEN during five weeks of horizontal bed rest. In contrast to the recent results they also found a decrease in fascicle architecture by 4.8% (FL). However, care must be taken when interpreting these results because architectural appearance is inhomogeneous over the muscle volume and measurements were taken over a limited area and probably not exactly above the same area pre and post. Furthermore, measurements of muscle architecture assume that fascicles are oriented in the image plane. In fact, however, fascicles run at an angle to the image plane (Bolsterlee et al. 2015).

In all considerations involving MVC, however, it should be remembered that the maximum contraction value of subjects before and after bed rest is not the same. In general, muscle force is lower due to the influence of long-term bed rest. Therefore, MVC was re-determined after bed rest and the gradations were related to the new value. However, the fascicle architecture has also changed for 0% MVC to approximately the same extent as for the measurements with contracted muscle. Since the MVC after bed rest obviously reflects the actual strength of the subjects at that time, no further adjustment or scaling was applied.

**FASCICLE EXCURSION DURING VIBRATION.** The main aim of the present study was to evaluate vibration-induced changes in fascicle length and pennation angle in gastrocnemius medialis depending on vibration frequency and muscular preload, as well as the influence of bed rest and artificial gravity as countermeasure. The main findings were a) that fascicle excursion (FL and PEN) decreased when the muscle is contracted, b) that fascicle excursion in contracted GM is greater during vibration with frequencies in the range from 7 to 10 Hz, c) that excursion was greater for 9 and 10 Hz (FL)

and 8, 9 und 10 Hz (PEN), respectively, after bed rest and d) that overall fascicle excursion was smaller in iAG countermeasure group after bed rest.

Mean FL excursion was greatest at 4 Hz vibration frequency in relaxed muscle, having a mean value of approximately 2%. Greatest total PEN excursion was 2.5% occurring at 16 Hz and relaxed muscle. Cochrane et al. (2009) previously reported an elongation of the gastrocnemius muscle tendon complex by 1° of its total length during 6 Hz vibration with 50% of the elongation occurring within the muscle itself. In the present study, excursion of fascicle length was 1.8% during 6 Hz vibration, giving an elongation by 0.9%. Even if the comparability of the studies is limited due to different methods and study designs, at least comparable magnitudes can be seen in the results.

As mentioned previously, overall excursion of FL and PEN was smaller in contracted muscle across frequencies than in muscle at rest. This can be attributed to an increase in stiffness when muscles are activated. The main parameter to quantify stiffness of soft tissues is the Young's modulus (Fung 1981). A greater Young's modulus is associated with a greater resistance of the material or tissue against elastic deformation. It is reported that Young's modulus is around 5-40 kPa in resting muscle and up to 300 kPa with contraction (Shinohara et al. 2010). Furthermore, Wakeling and Nigg (2001) reported that damping coefficient increased by the force produced by the muscle. These muscle properties may explain a decreased fascicle excursion in contracted muscle compared to relaxed muscle. However, it appears that the extent of contraction is less important than whether the muscle is contracted at all or not, at least at the levels of MVC studied here.

The effect described above is additionally overlaid by a frequency-dependent increase in fascicle excursion. For vibration frequencies from 7 to 10 Hz there are significant increases in excursion for pre-tensioned muscle, especially for fascicle length (Fig. 10). This increase in excursion within a small range of frequencies may be interpreted as a phenomenon of resonance. Soft tissue is expected to resonate if the excitation frequency of a mechanical stimulus is close to the natural frequency of the tissue (Wakeling et al. 2002). Natural frequencies of triceps surae, quadriceps and tibialis anterior were previously described to range from 10 Hz from relaxed condition to 50 Hz for maximal contracted state (Wakeling and Nigg 2001). If it is considered that the muscle-tendon complex acts as a spring-mass system, the natural frequency ( $\omega_0$ ) of such a system can be described as  $\omega_0 = \sqrt{k/m}$ , where  $k$  is stiffness and  $m$  is mass (Rittweger 2010). As mass usually remains unchanged, changes in natural frequency must be caused by modification of stiffness. Therefore, the natural frequency would have to increase by the factor of  $\sqrt{2}$  when stiffness is doubled. Lambertz et al. (2001) reported best fit for the stiffness-torque relationship to be linear, but when concerning whole MTU stiffness. The

results of Hug et al. (2015) also confirm that muscle stiffness is linearly related to muscle forces. Consequently, if the resonant frequency was exclusively dependent on the stiffness and stiffness increases linearly with force, an alteration of the resonant frequencies toward higher frequencies is expected to occur with increasing contraction. However, in the present results there was no significant alteration of the frequency at which the excursion maxima occur across the levels of contraction (25%, 50%, 75% MVC). Therefore, both a centroid of area calculation and a cubic spline approximation in the range from 7 to 12 Hz were used to determine the vibration frequency with maximum excursion, but the resulting values remained around 8 and 9 Hz. One possible explanation for a constant resonance frequency is that damping properties increase to the same extent as stiffness when the muscle activity elevates. This hypothesis is accompanied by the results of Wakeling and Nigg (2001), who reported that the damping coefficient increased by the force produced by the muscle. It is therefore conceivable that the resonant frequency remains unchanged if the influence of damping and stiffness in the muscle is equivalent.

A further explanation for the resulting resonance effect includes the physiological tremor. In general, it is assumed that physiological tremor is either a movement appearing due to oscillations within the central nervous system or it is simply the consequence of mechanical properties of the limb. Lakie (1994) has shown that the acceleration spectrum of the physiological tremor has a significant peak between 7 and 11 Hz. It is therefore conceivable that the increase in excursion at vibration frequencies in the range from 7 to 10 Hz in the present study is a consequence of resonance within the natural tremor frequency. In fact, it is possible that the induced vibration causes the physiological tremor to resonate. Nevertheless, an explanation still has to be given why 1) the resonance frequency did not shift and why 2) the resonance does not appear at 0% MVC. On the one hand it has been demonstrated that tremor frequency increased from 6.5 Hz to 7.5 Hz when additional load on the hand is applied due to an increased gravitational force from 1 g to 2.5 g (Lakie et al. 2015). This can also be attributed to an increased stiffness due to an elevated muscular effort. On the other hand, this elevated stiffness is mitigated by the compliance of series tendon. Both, the small increase in tremor frequency when additional load is applied and the mitigated stiffness in the MTU could possibly explain why the resonance frequency in the present results remained unchanged. To explain 2), the amplitude (acceleration) of the tremor must be incorporated. When gravitational field strength was increased up to 2.5 g in the study mentioned above, the acceleration of tremor was approximately four times greater compared to 1 g. Consequently, it is conceivable that tremor size and thus the amplitude of resonance was only large enough to be significantly recognizable when additional load was applied

(muscle contraction). Overall, the excitation of the physiological tremor within its resonant frequency range is a possible explanation for the increased fascicle excursions in the present results. For further investigations and more detailed explanations it is important to study the latency between actuator (i.e. MARES) and the muscle movement, which allows further insight into the mechanical properties of muscle, especially stiffness.

The excursion after bed rest was greater at 9 and 10 Hz for FL and at 8, 9 and 10 Hz for PEN. Consequently, resonating effects seem to occur even more clearly due to the influence of bed rest. This indicates that the muscle reacts with an increased excursion when excited within a certain range of frequencies. As there are no significant increases in excursion for other frequencies after bed rest, the effect cannot be explained solely by an alteration of stiffness of the muscle.

The iAG-group was the only group in which significant time effects were observed for fascicle length. Here excursion values were smaller post bed rest than baseline. A likely explanation is that intermittent centrifuge drives influenced the mechanical properties in such a way that muscle stiffness increased. On the other hand, it should be noted that these are relative values in relation to the total fascicle length measured before vibration started. In iAG group, fascicle length values were greater for R5, which results in smaller relative excursion values. Nevertheless, there are several other changes in muscular tissue caused by bed rest that affect the extent of fascicle excursion, such as a decrease of mass or changes in elastic properties of tendons. These alterations and their influence on fascicle excursion require further investigation.

In addition to the limitations already mentioned, it should be remembered that both the quality of the ultrasound videos and the accuracy of fascicle tracking are limited. It is therefore possible that the detection of fascicles was subjected to inaccuracies due to low image quality. Also, the tracking of the fascicles may be affected by certain tracking errors, especially in case of large changes during highly dynamic movements. Nevertheless, the results obtained show reliable and promising results.

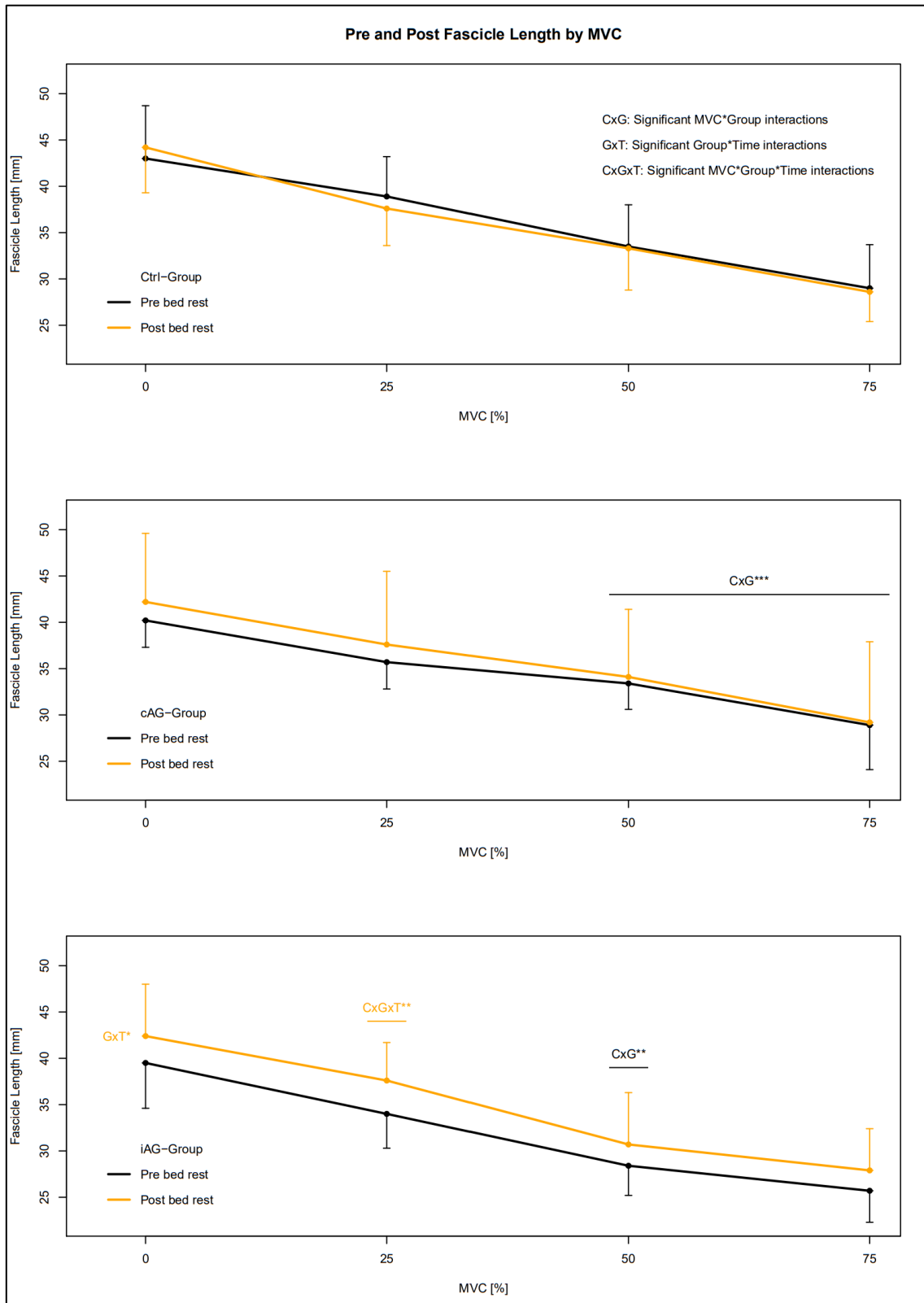
This study is the first to describe changes in fascicle length and pennation angle in human gastrocnemius muscle during vibration and muscular preload and provides an initial overview of the extent of fascicle excursion at different frequencies and levels of submaximal isometric contraction. It is hoped that these results will prove useful to future studies investigating the influence of vibration on the muscle architecture or muscle tendon unit.

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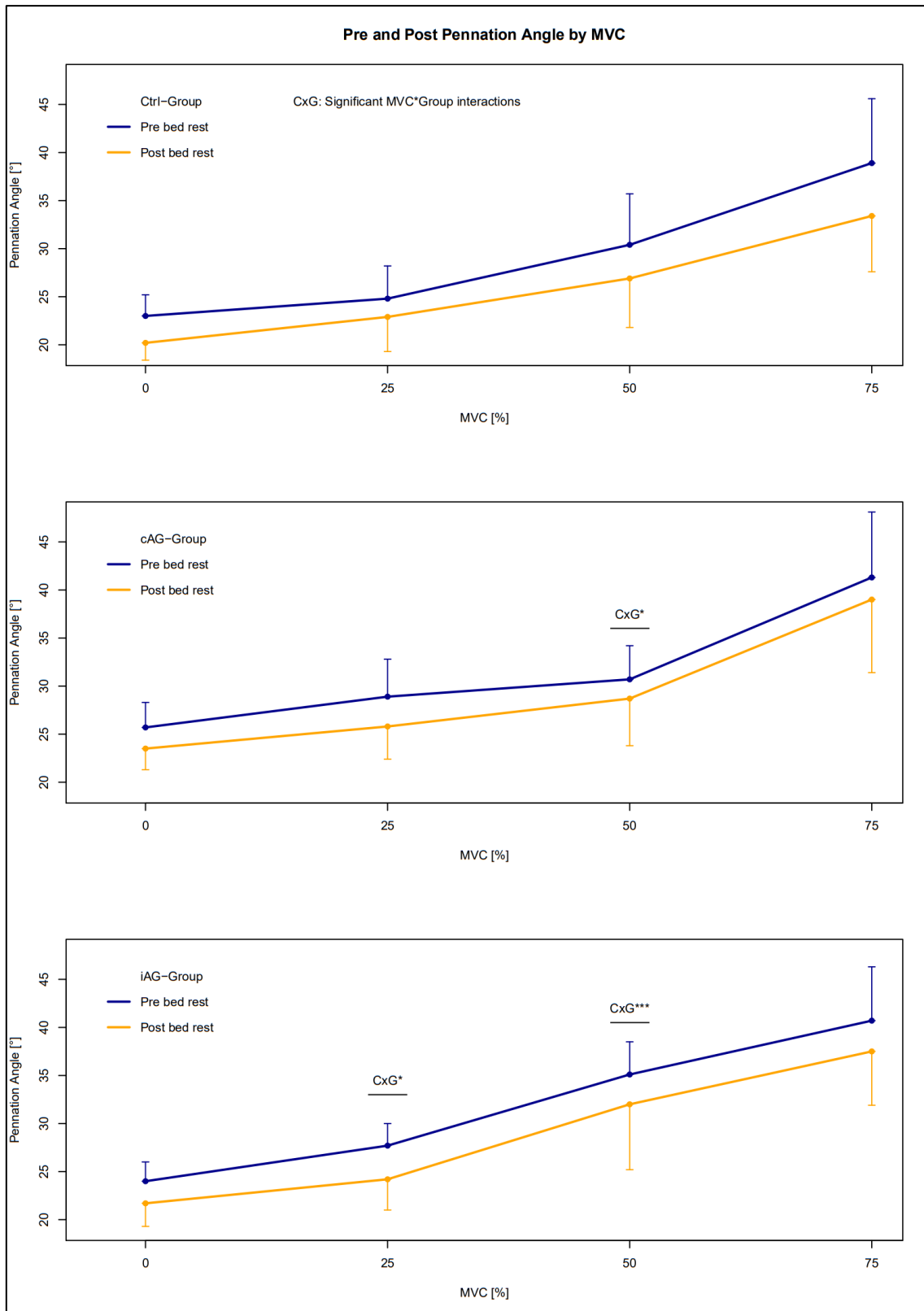
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APPENDIX

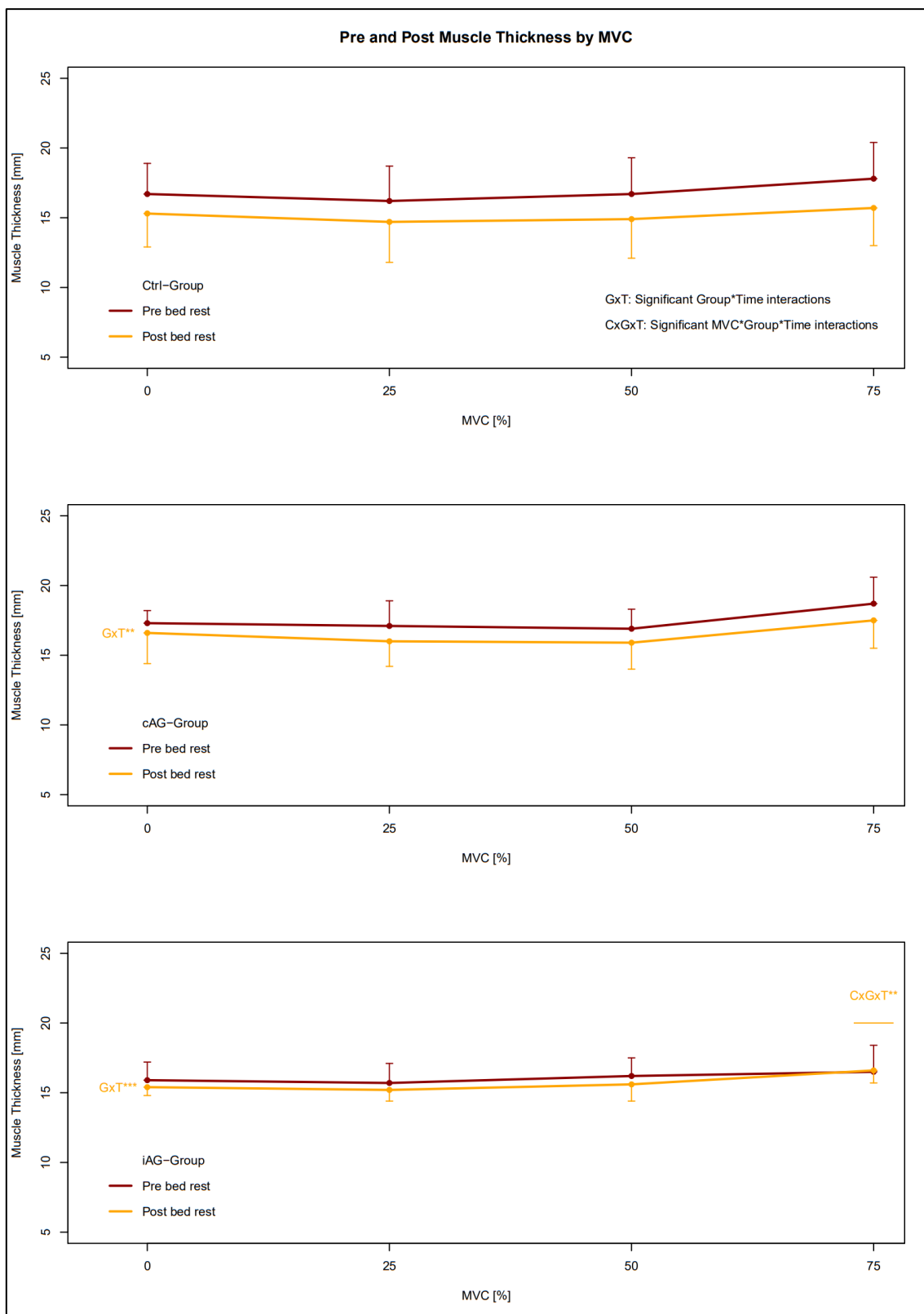


**Figure 9.1. Mean (SD) fascicle length pre and post bed rest by groups.** CxG denotes significant MVC × group interaction with 0% MVC × Ctrl-Group as reference level of contrast. GxT denotes significant group × time interaction, reference level of contrast being Ctrl-Group × BDC. CxGxT denotes significant MVC × group × time interaction effect with 0% MVC × Ctrl-Group × BDC as level of contrast. (\*\*\*)  $P < 0.001$ , (\*\*)  $P < 0.01$ , (\*)  $P < 0.05$ ).

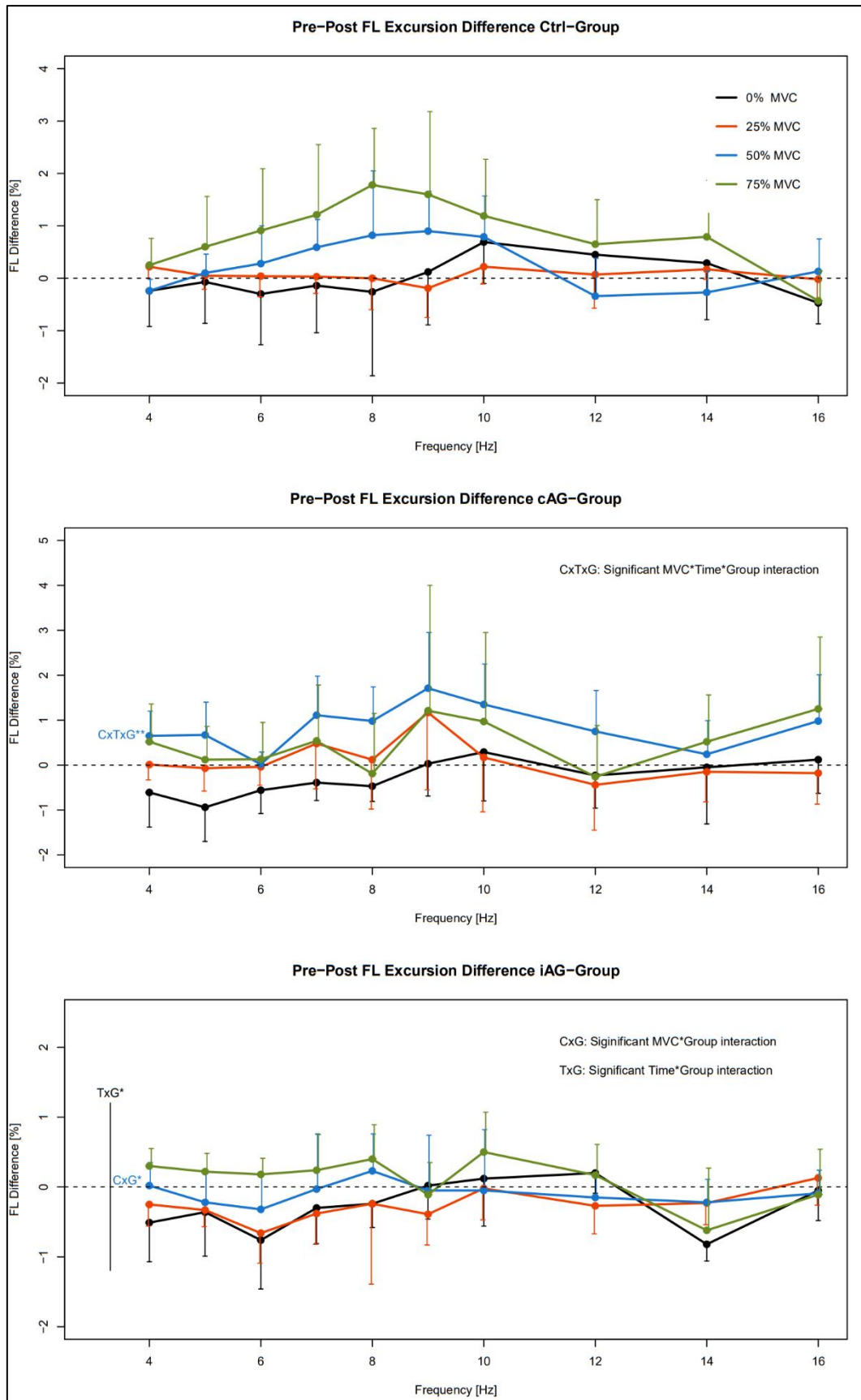




**Figure 9.2.** Mean (SD) pennation angle pre and post bed rest by groups. CxG denotes significant MVC × group interaction with 0% MVC × Ctrl-Group as reference level of contrast. (\*\*\*)  $P < 0.001$ , (\*\*)  $P < 0.01$ , (\*)  $P < 0.05$ .



**Figure 9.3. Mean (SD) muscle thickness pre and post bed rest by groups.** GxT denotes significant group × time interaction, reverence level of contrast being Ctrl-Group × BDC. CxGxT denotes significant MVC × group × time interaction effect with 0% MVC × Ctrl-Group × BDC as level of contrast. (\*\*\*) P < 0.001, (\*\*) P < 0.01, (\*) P < 0.05).



**Figure 12.1. Mean (95% CL) FL excursion difference between post and pre bed rest by group.** CxG (CxTxG) denotes significant interaction effects for MVC × group (MVC × time × group), with 0% MVC × Ctrl-Group (0% MVC × BDC × Ctrl-Group) as reference level of contrast. TxG denotes significant time × group interaction, reference level being BDC × Ctrl-Group. Color represents corresponding MVC level. (\*\* P < 0.01, \* P < 0.05).

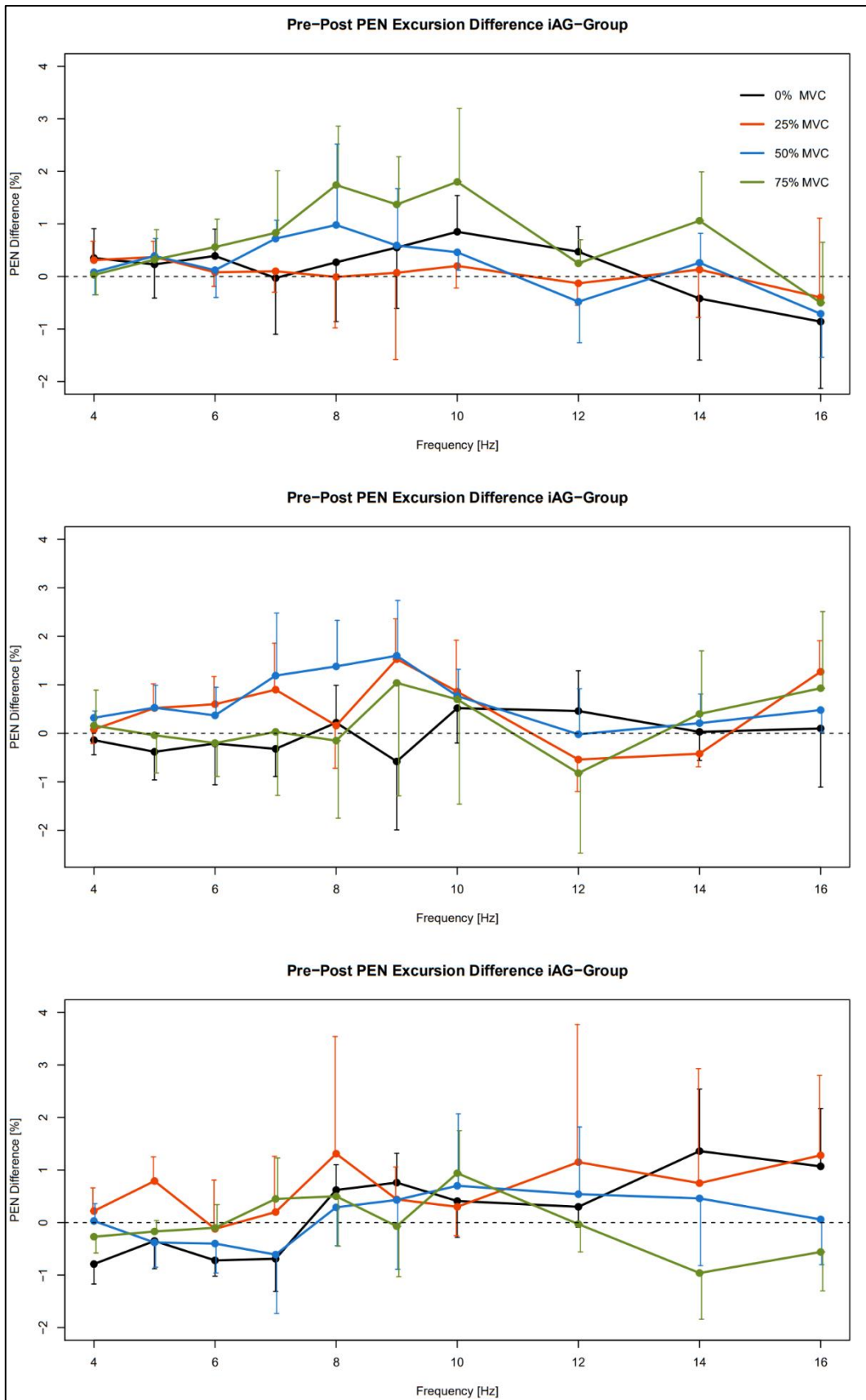


Figure 12.2. Mean (95% CL) PEN excursion difference between post and pre bed rest by group.