Effect of Whole-body Vibration frequency on muscle tensile state during graded plantar flexor isometric contractions

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ABSTRACT

Background: Acute physiological and biomechanical alterations have been reported following whole-body vibration (WBV). Stiffening of muscles has only been anecdotally reported in response to WBV. Accordingly, this study investigated active plantar flexor muscle stiffness in response to a single WBV bout at four mechanical vibration frequencies.
Methods: Thirteen healthy adults (37.1 ± 14.4 years old) randomly received WBV in 4 different frequencies (6, 12, 24, and 0 Hz control) for 5 min. Shear wave speed (SWS) in longitudinal and transverse projections, architecture, and electric muscle activity were recorded in the medial gastrocnemius (MG) and soleus (SOL) muscle during graded plantar flexor contraction. Subjective rating of perceived muscle stiffness was assessed via Likert-scale.
Results: SWS of the MG at rest was enhanced in response to 5 min of 24 Hz WBV ($p = 0.025$), while a small reduction in SOL SWS was found during contraction ($p = 0.005$) in the longitudinal view. Subjective stiffness rating was increased following 12 Hz intervention. After 24 Hz WBV, pennation angle for MG was decreased ($p = 0.011$) during contraction. As a secondary finding, plantar flexor strength was significantly increased with each visit, which, however, did not affect the study's main outcome because of balanced sequence allocation.
Conclusion: SWS effects were solely limited to 24 Hz mechanical vibration and in the longitudinal projection. The observed effects are compatible with an interpretation by post-activation potentiation, warm-up, and force-distribution within the triceps surae muscles following 5 min WBV. The outcome may suggest SWS as a useful tool for assessing acute changes in muscle stiffness.

1. Introduction

Whole-body vibration (WBV) has been gaining popularity and is now widely used as part of rehabilitation and strength training[1] in a wide range of health conditions, such as, chronic ankle instability,[2] post-menopausal women,[3,4] older adults.[5,6] It has been demonstrated that mechanical vibration induces cyclical stretch-shortening in the active muscle and its tendon which can be useful for muscle performance facilitation.[7] The fact that electromyographic activity may be modulated at the same frequency as the vibratory stimulation[7–9] suggests that the vibration-induced muscle-tendinous elongation might elicit stretch reflexes,[8,10,11] although this interpretation was recently questioned.[12,13] Several studies have shown increased contractile strength and rate of force development after an acute bout of WBV with amplitude and frequency in the range of 3.5–6.0 mm and 26–35 Hz, respectively[14–16]. Although, some adverse effects could be found following high-frequency WBV application, including muscle fatigue, muscle soreness, and paresthesia.[17] Rittweger and colleagues[18] also suggested that less energy transfer and reduced acceleration in using low-frequency WBV may be an advantage for safety and decreased the impact on muscle activity compared to high-frequency WBV uses. A recent finding has also

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Abbreviations

FL	Fascicle length
LG	Lateral gastrocnemius
MF	Median frequency
MG	Medial gastrocnemius
MVC	Maximum voluntary contraction
PA	Pennation angle
PF	Plantar flexion
RMS	Root-mean-squared
ROI	Region of interest
SOL	Soleus
SWE	Shear wave elastography
SWS	Shear wave speed
TA	Tibialis anterior
WBV	Whole-body vibration

reported that low-frequency WBV during squat exercises in the range of 4–16 Hz significantly increased the medial gastrocnemius activity in healthy young adults.[19] However, there is to date no scientific evidence for the stiff-sensation perception in relation to the frequency of WBV employed. No objective assessment of muscle stiffness has been made after mechanical vibration exposure, but trends can be observed by looking at average electromyography (EMG) responses.[20]

A number of studies has discussed possible reasons for the changes in muscle mechanical properties induced by WBV with a peak to peak amplitude of 6 mm and frequency of 26 Hz for 6 min[17] and 1 min.[21] Flexibility or passive stiffness improvement is substantially useful for patients as a part of warm up activities and rehabilitation programs.[22,23] Stiffness changes have, however, been studied as a function of chronic response to mechanical vibration. Thus, a study of Lapole and Pérot[24] using a quick-release test, found that musculotendinous passive stiffness of the triceps surae was significantly decreased after 14 days of 1 h Achilles tendon vibration at 50 Hz with 0.2 mm amplitude. However, Cronin and co-worker[25] found that 5 × 60 s with 1 min rest in between of one leg vibration at 26 Hz, amplitude 6 mm did not change plantar flexor muscle stiffness determined by a damped oscillation technique and similar negative findings are reported by the previous studies using 8 mm amplitude, at 26 Hz or 3 mm amplitude, at 40 Hz for 6 min long with 1 min rest in between [22] and, using a frequency of 30 Hz with 2 mm amplitude for 10 min with 1min rest,[26] albeit looking at different structures with varying method. From a physiological point, vibration-related stretch-shortening cycles can potentially result in stretch-mediated force enhancement[27] but also in shortening-mediated force depression.[28] Both of these effects are speed- and time-dependents. Moreover, vibration leads to post-activation potentiation[14] and modulation of spinal and supra-spinal reflexes.[29] Given the time-dependency of all these processes, it seems well possible that stiffening and relaxing effects may depend on the vibration frequency.

The aforementioned muscle stiffness can be detected by using a variety of methods, such as damped-oscillation,[25] ultrasound-indentation,[30] or hopping-based assessment of stiffness.[31] However, all of these approaches were investigated on adjacent structures. Ultrasound shear wave elastography (SWE) is a novel method that can also be selectively applied to muscle,[32,33] and muscle stiffness increases seem to be associated with shear-wave speed (SWS) increases.[34] Muscle stiffness is related to both active and passive muscle forces and muscle stiffness measurement via SWE has been used to estimate changes in active muscle force with the level of activation.[32] It can also be used to distinguish between passive and active force generation.[35] SWS can be performed in a consistent manner by controlling transducer pressure, tissue depth, transducer position relative to longitudinal muscle fibres, region of interest, size, etc.[36] Interestingly, SWE has also

been used to examine changes in passive stiffness following WBV. In a recent study,[37] it was demonstrated that 15 min of local mechanical vibration did not alter passive knee extensor muscle stiffness. In another study with SWE, Pournot et al.[38] showed no beneficial effect of local 10 min mechanical vibration at 55 Hz on the recovery of exercise-induced stiffness of the biceps brachii. The effect of mechanical vibration on active muscle stiffness has not been assessed. We therefore ventured to assess the acute response in active plantar flexor muscle stiffness to a single bout of WBV of various frequencies by using SWE. The main hypothesis is that mechanical vibration affects longitudinal SWS in muscles at rest. We further hypothesize that muscle stiffness is also affected during contractions, that higher frequency WBV would have a differential effect on muscle stiffness compared to lower frequency, and that the WBV effect would be different in longitudinal and horizontal planes.

2. Materials and methods

2.1. Participants

In total, 6 healthy males and 7 females were recruited (37.1 ± 14.4 years; body mass index (BMI) 22.8 ± 2.4 kg/m^2). They were aged between 18 and 65 years who had BMI between the range of 10–20 kg/m^2 for men and 18–28 kg/m^2 for women. Exclusion criteria were neuromuscular, vestibular or skeletal problems associated with balance or contraindications to exercise. After the procedures had been carefully explained, the participants provided written informed consent to the study that was approved by the ethical committed of the Landesärztekammer Düsseldorf Ethics Committee (Ref.#2018332).

2.2. Study design

This study was a controlled, cross-over (balanced) design with CONSORT diagram (Fig. 1). An initial session familiarized the participants with the equipment and experimental procedure. Participants attended a total of 4 sessions, and a different WBV frequency was randomly tested during each session. The frequencies were either 0 (Control), 6, 12 or 24 Hz separated by at least 1 day,[18] and they were administered in a balanced sequence by using computerized blocks of six that were randomly assigned to each participant. The whole study procedure is shown in Fig. 2. Upon arrival, subjects warmed up at 2 W/kg on a bicycle ergometer for 10 min, following by the baseline maximum voluntary contraction (MVC) force tested. Thereafter, a pre-WBV graded plantar flexor (PF) contraction test (Fig. 3A) was done during which elastography and muscle architecture measurements of the medial gastrocnemius (MG) and soleus (SOL) muscles along with
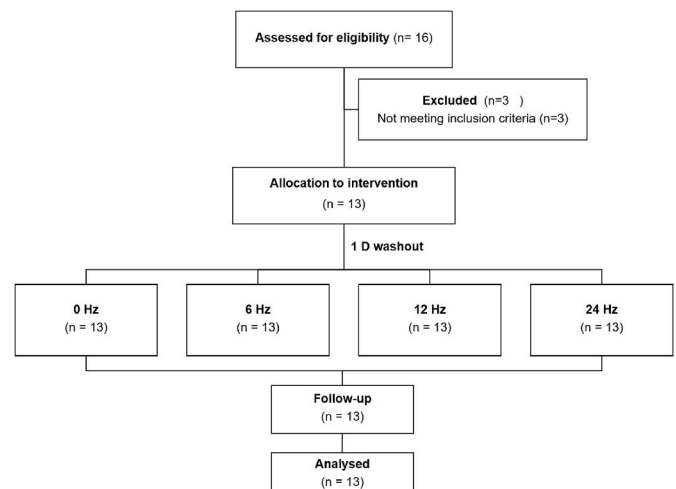

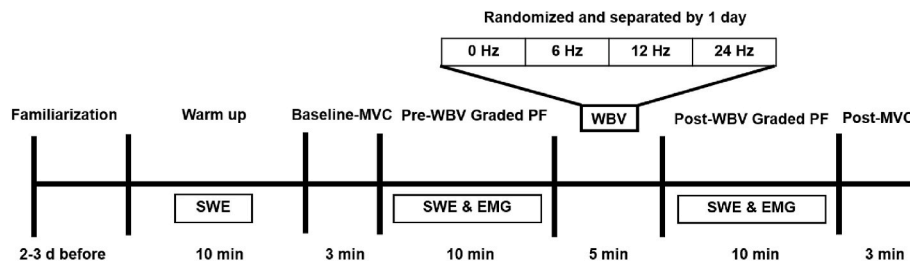**Fig. 1.** CONSORT diagram of the study.

Fig. 2. The study timeline before and immediately after whole-body vibration (WBV). MVC, maximum voluntary contraction. PF, plantar flexion. SWE, shear wave elastography. EMG, electromyography.
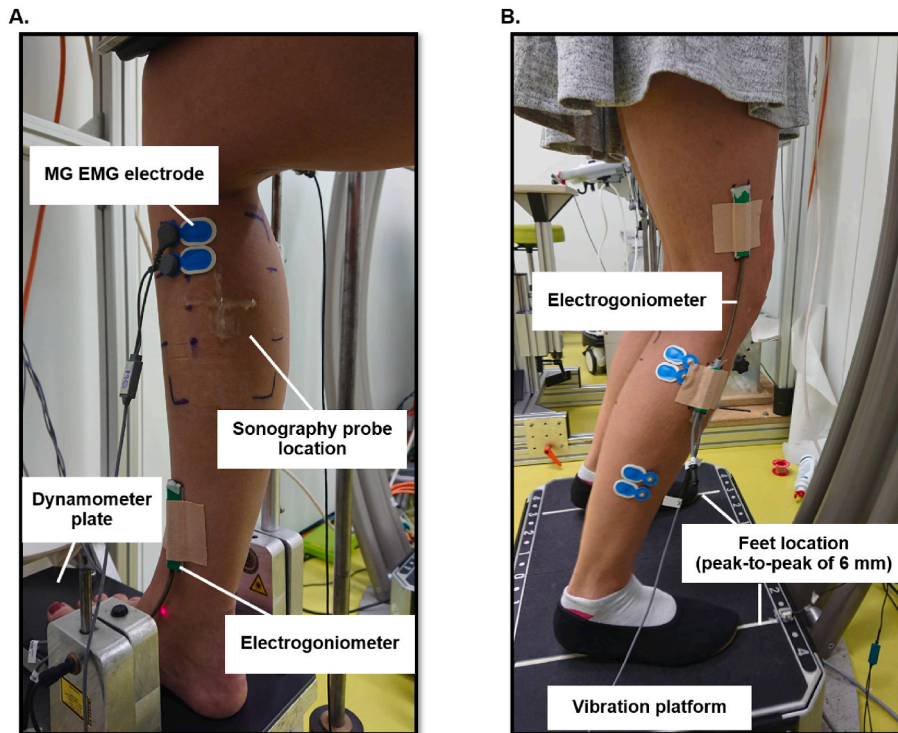

Fig. 3. Experimental set up. A. During graded plantar flexor contraction test. B. During WBV procedure.

muscle activities of MG, SOL, lateral gastrocnemius (LG) and tibialis anterior (TA) were assessed. Subjects then stood on a mechanical vibration platform for 5 min with a selected vibration frequency (Fig. 3B). After this, the post-WBV graded plantar flexor contraction test was assessed again and finally post-MVC was determined. All measurements were carried out by the same investigator (PM).

2.3. Graded plantar flexor contraction test

The test was performed under isometric conditions. Participants were seated in a custom-made hard-back chair with the right knee in 90 deg flexion and the right foot placed on a dynamometer plate (Fig. 3A). The right medial/lateral malleolus was marked and aligned with a laser pointer being the point of rotation. In the first visit, participants' leg positioning and restraints upon the leg were recorded prior so that the subject could be repositioned in the chair in the exact same position after WBV in all subsequent visits. The investigator positioned the restraint on the thigh such that the participant could not lift their heel, but no force was generated on the foot plate. The participant was then asked to perform a maximum voluntary plantar flexion (PF) contraction (baseline-MVC). Following this, three elastography readings were measured separated by 5 s with the muscle at rest, 25, 50, and 75% of maximum contraction strengths (pre-WBV graded PF in longitudinal view and

transverse view, respectively Fig. 4) while the participant held the force constant with the support of a visual feedback system for 3 s. Rest breaks for 1 min were allowed between contractions (where muscle was completely relaxed) along with EMG muscle activities of four muscles detected. After 5 min WBV, the post-WBV graded PF was repeated with the same absolute forces as used as the baseline-MVC to compare the SWS and contractile force relationship and then the post-MVC of plantar flexor muscle after WBV was recorded.

2.4. Ultrasound imaging

A real-time B-mode computerized ultrasound system with sonography (9L probe, LOGIQ S8, GE Healthcare, USA) with a linear array probe of 7.5–12 MHz wave frequency was used to obtain longitudinal and transverse ultrasonic images of right MG and SOL muscle during MVC and graded plantar flexor contraction test. The probe was positioned with a 2 mm gel coat at the most prominent bulge of the MG muscle at 1/3 of the line between the head of the fibula and the heel using a foam fixation holder. During the test, a square region of interest (ROI) was defined for the elastography function such that the ROI was completely contained within the middle part of MG and underneath SOL muscle without vessels included. The ROI was the same size and location on each testing day. To ensure the accuracy of the measurements, the
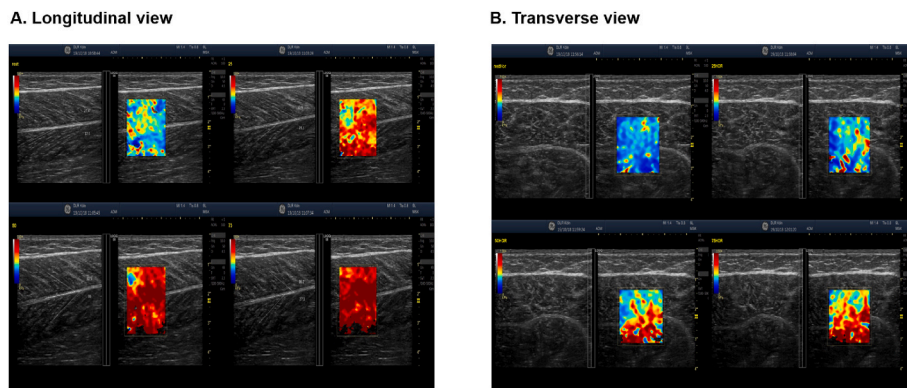
A. Longitudinal view   **B. Transverse view**

Fig. 4. An example of participant No. 1's SWE image during graded plantar flexor contraction test (at resting, 25%, 50%, and 75% of MVC). A. Image in longitudinal view of MG and SOL. B. Image in transverse view of MG and SOL.

vertical probe position was adjured to best align the probe signal relative to the muscle fascicles and the lightest transducer pressure was used because of anisotropy and deformability of muscle structure.[34,39] After images were taken with the longitudinal probe orientation, the ultrasound probe was gently turned to be perpendicular to the muscle in the same location and the images in this plane were captured during repeated graded plantar flexor contraction test at the same force levels.

Participants were advised to relax and the EMG signal was monitored while collecting resting images before and 5 min after WBV the elastography images were taken for each level as described above in the same positions. A trace area that delineated the ROI for the measurement of SWE was placed in areas of the acquisition box on each the MG and SOL without deep fascia aponeurosis between them (Fig. 5). The mean average of SWS (in m/s) of 3 images on the MG and SOL in each state of contraction within the ROI were estimated by the built-in specific quantification program. The report of SWS in m/s was selected because of being the most appropriate stiffness unit for muscle.[40] From the longitudinal view images, pennation angle (PA) was assessed offline for MG and SOL during graded plantar flexor isometric testing analytically with custom-made python scripts. In addition, we were able to also assess fascicle length (FL) for MG, which was not possible for SOL because the deep aponeurosis was not clearly visible in many images.

2.5. Electromyography

EMG activity was recorded at a sampling rate of 1000 Hz using a Noraxon 1400A bio-recording system from the right leg of the MG muscle, LG muscle, SOL muscle and TA muscle, using the guidelines of the manufacturer of the EMG device during pre and post WBV graded plantar flexor isometric testing. Surface pre-gelled Ag–AgCl electrodes, 10 mm diameter (Medicostest, Rugmarken, Denmark) were placed over the muscle belly at an inter-electrode distance of 20 mm with a reference electrode placed over the head of the fibula. The skin of the electrode site was prepared by shaving, gentle abrasion using a gel-based product (Everi, Spes Medica, Italy), and cleansed with an alcohol swab. The EMG amplitude was visualized to take into account any contraction movement while resting SWE images were taken. For off-line analysis, EMG data were band-pass filtered (cutoff: 10–500 Hz) with a 4-pole Butterworth filter, and root-mean-squared (RMS) amplitude and median frequency (MF) was assessed during the PF contraction. RMS amplitude during the graded PF contractions were then normalized to the RMS amplitude values during MVC and expressed in percent (percRMS).

2.6. Whole-body vibration

WBV was performed on a commercial machine (Galileo Sport, Novotec, Pforzheim, Germany), which has a motorized teeterboard that produces side-to-side alternating vertical sinusoidal mechanical vibration to the body. During the exposure to WBV, the participants stood barefoot on the platform to eliminate any mechanical vibration damping caused by footwear (Fig. 3B). Feet were placed on either side of the central axis which corresponded to mechanical vibration with peak-to-
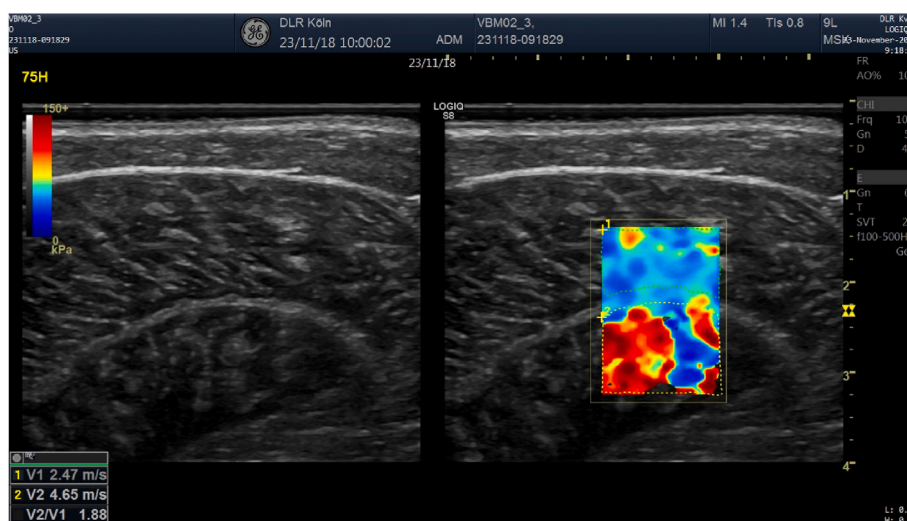

Fig. 5. Defining the region of interest (ROI) for the measurement of SWS placed all areas of the acquisition box on each the MG and SOL and SWS was shown in the bottom left corner in m/s.

peak displacement of 6 mm, and the vibration frequency was randomly set to either 0, 6, 12 or 24 Hz on different occasions separated by at least 1 day. Participants stood in a static squat position of 20° knee flexion with heel slightly raised[14,41,42] for 5 min in which induced post-activation potentiation.[14] An electrogoniometer (Model SG110, Biometrics Ltd, Gwent, UK) was used to set the knee angle, and to ensure that the position was maintained throughout the intervention. The participants were asked to simultaneously maintain their trunk as upright as possible and keep their center of pressure above the forefeet; for safety, they remained next to a handrail in case they lost their balance. Immediately after 5 min WBV, they were asked and noted any adverse effect which might present such as, muscle fatigue, muscle soreness, and paresthesia.[17]

2.7. Rating of perceived muscle stiffness

The subjective rating of calf muscle stiffness after WBV was given by the participants immediately after the 5 min mechanical vibration using a Likert-scale ranging from +3 to −3, where +3 represented "strongly stiff" and −3 "very relaxed".

2.8. Statistical analysis

Sample size estimation was based on the hypothesis that mechanical vibration exposure would affect longitudinal muscle SWS at rest. It was considered that recruitment and testing of 13 subjects would be well feasible in the time-frame for which the lab was accessible for this study, Preliminary data had yielded a mean value of 1.81 m/s and a SD of 0.036 m/s for the muscle tissue's SWS, and a power analysis (function pwr.t.test of the library 'pwr' of the R-environment) suggested that the planned sample size of 13 participants could pick up effects <1%, which seemed suitable to detect clinically relevant changes. Thus, the study was powered for the primary hypothesis only, and all other, exploratory hypotheses were tested without adjustment for multiple comparisons.

Where normally distributed, differences such as between pre and post WBV are given as mean and 95% confidence interval. Main and interaction effects of SWS were determined by 3-way repeated-measures ANOVA [muscle (MG, SOL) x force level (0, 25, 50, 75, 100%) x probe orientation (longitudinal, transverse)] and where significant interactions were present, post hoc multiple comparisons were made, with Bonferroni correction and no significant three factor interaction effects were found. The most important comparison in the present study was the effect of mechanical vibration frequency on SWS (primary outcome). Therefore, changes in the SWS over time (before and after mechanical vibration uses) were compared between % force contraction level for each muscle (MG and SOL) by two-way ANOVA and, likewise, for the probe orientations. Where significant effects were found, post hoc testing was performed using paired t-tests with Bonferroni correction for multiple comparisons. The statistical approach for the secondary outcomes (muscle architecture and EMG signal) were analyzed by 4-way

repeated measures ANOVA, 2-way repeated measures and paired t-test was analyzed for subjective muscle stiffness rating assessment. An alpha level of 0.05 was used to determine statistical significance. All statistical analyses were performed by using Statistic Package for the Social Sciences (SPSS for Window version 22.0, Chicago, IL, USA).

3. Results

All 13 subjects participated in every testing session without any adverse effect. Maximal plantar flexor force baseline was comparable across WBV frequencies ($p > 0.05$, Table 1), but differed between visits F $(3,33) = 5.2$, $p = 0.005$; $\eta^2 = 0.32$, being smallest at visit 1 and largest at visit 4. After 10 min of warm-up, longitudinal SWS was unchanged as compared to before the warm-up, being on average 2.19 ± 0.3 m/s in MG and 2.57 ± 0.6 m/s in SOL compared before warm up 2.18 ± 0.2 m/s in MG and 2.47 ± 0.5 m/s in SOL ($p > 0.05$). However, maximal plantar flexor torque was elevated after WBV for all vibration frequencies (F$(1,11) = 189.8$, $p < 0.001$; $\eta^2 = 0.95$), including 0 Hz and in every visit order (F$(1,11) = 187.8$, $p < 0.001$; $\eta^2 = 0.95$). This effect was independent of the vibration frequency (F$(3,33) = 0.72$, $p = 0.062$; $\eta^2 = 0.062$) as shown in Table 1.

3.1. Shear wave speed assessment

Data for SWS of MG and SOL during graded plantar flexor contractions both before and after WBV are given in Table 2. The main hypothesis was tested by 2-way ANOVA independently for SOL and for GM at rest, both in longitudinal and in horizontal projection. No time frequency interaction effects were found (all $p > 0.20$). With all these conditions assessed by a 3-way repeated-measures ANOVA approach regardless of vibration frequency, SWS was higher in SOL than MG (F $(1,10) = 47.2$, $p < 0.001$; $\eta^2 = 0.83$). SWS increased with contraction level (F$(4,40) = 65.1$, $p < 0.001$; $\eta^2 = 0.87$), and SWS was greater in longitudinal view as compared to transverse view (F$(1,10) = 8.3$, $p = 0.016$; $\eta^2 = 0.45$). No significant three factor interaction effects were found (F$(4,40) = 1.9$, $p = 0.136$; $\eta^2 = 0.16$).

Two-way repeated ANOVA yielded an interaction effect between time and frequency on SWS seen after WBV in MG at 25 % of force level from the transverse view (F$(3,36) = 3.2$, $p = 0.036$; $\eta^2 = 0.21$), indicating an insignificantly increased SWS after 0 and 24 Hz, but insignificantly decreased SWS after 6 and 12 Hz of WBV. Another interaction effect was found between the time and contraction force level for MG at 0 Hz WBV in the longitudinal projection (F$(4,48) = 3.3$, $p = 0.018$; $\eta^2 = 0.22$). Moreover, with post hoc analysis, there was an increase in MG longitudinal SWS 0.36 m/s (0.02–0.70 m/s) after no intervention (0 Hz) ($p = 0.040$, Fig. 6A). A higher resting longitudinal SWS of MG was found significantly with the mean difference 0.21 m/s (0.03–0.38 m/s) after 24 Hz WBV ($p = 0.025$, Fig. 6D) and there was only a small decrease in SOL longitudinal SWS (F$(1,12) = 11.8$, $p = 0.005$; $\eta^2 = 0.50$) after 24 Hz of WBV at 25 % of MVC being 4.12 m/s (3.30–4.95 m/s) ($p = 0.026$)

Table 1
Results for contraction force (in N) during maximal voluntary contractions (MVC) in plantar flexion (PF), given per vibration frequency (upper part) and per visit (lower part).

| | WBV frequency (Hz) | | | |
	0	6	12	24
Baseline MVC (N)	627.2 ± 134.8	608.0 ± 119.7	617.4 ± 111.0	622.1 ± 124.5
Post-MVC (N)	652.7 ± 145.0*	634.7 ± 118.7*	638.7 ± 113.1*	637.1 ± 123.5*
	Visit number			
	1st	2nd	3rd	4th
Baseline MVC (N)	587.7 ± 116.4†	618.7 ± 128.9	620.8 ± 129.2	644.9 ± 111.2
Post-MVC (N)	608.7 ± 117.0*	636.8 ± 133.5*	648.5 ± 134.9*	669.0 ± 109.3*

PF; plantar flexion, WBV; whole-body vibration. Data are means and SD. * Significant changes from before standing on WBV ($p < 0.001$). † Significant difference from the subsequent visit for baseline MVC ($p < 0.001$).

Table 2
SWS after WBV for both MG and SOL muscles during graded plantar flexor contraction.

Muscle	Vibration frequency	Projection	Shear wave speed (m/s)									
			Pre					Post				
			Contraction level (% MVC)					Contraction level (% MVC)				
			0	25	50	75	100	0	25	50	75	100
MG	**0 Hz**	Longitudinal	2.14 ± 0.3	2.49 ± 0.6	2.84 ± 0.7	3.94 ± 1.0	4.92 ± 1.0	2.17 ± 0.3	2.85 ± 0.6*	3.21 ± 0.7	3.78 ± 1.0	4.92 ± 1.0
		transverse	2.02 ± 0.4	2.24 ± 0.4	2.4 ± 0.4	2.93 ± 0.5	3.97 ± 1.1	1.93 ± 0.2	2.33 ± 0.4	2.57 ± 0.6	3.21 ± 1.0	4.22 ± 1.4
	6 Hz	Longitudinal	2.29 ± 0.7	2.60 ± 0.8	2.98 ± 0.8	3.78 ± 1.0	5.07 ± 1.3	2.18 ± 0.3	2.46 ± 0.4	2.86 ± 0.7	4.01 ± 1.3	5.19 ± 1.4
		transverse	2.21 ± 0.4	2.32 ± 0.4	2.63 ± 0.6	3.13 ± 0.7	4.18 ± 1.5	2.09 ± 0.4	2.20 ± 0.5	2.37 ± 0.5	3.21 ± 0.9	6.61 ± 7.7
	12 Hz	Longitudinal	2.14 ± 0.3	2.66 ± 0.6	3.16 ± 0.7	3.84 ± 1.0	4.88 ± 1.1	2.07 ± 0.4	2.54 ± 0.6	3.06 ± 0.8	4.01 ± 1.0	5.08 ± 1.1
		transverse	2.41 ± 1.4	2.24 ± 0.5	2.53 ± 0.5	3.52 ± 1.2	4.38 ± 1.4	1.92 ± 0.3	2.13 ± 0.6	2.49 ± 0.6	3.35 ± 1.2	4.05 ± 1.1
	24 Hz	Longitudinal	2.19 ± 0.3	2.59 ± 0.6	3.07 ± 0.8	3.75 ± 1.2	5.26 ± 1.4	2.40 ± 0.2*	2.61 ± 0.8	2.78 ± 0.4	3.70 ± 1.2	5.06 ± 1.3
		transverse	1.97 ± 0.4	2.21 ± 0.4	2.47 ± 0.5	3.10 ± 0.9	4.20 ± 1.4	2.01 ± 0.3	2.33 ± 0.6	2.50 ± 0.5	3.68 ± 1.6	4.58 ± 1.6
SOL	**0 Hz**	Longitudinal	2.66 ± 1.2	4.56 ± 1.2	5.29 ± 1.1	5.96 ± 1.2	6.19 ± 1.2	2.54 ± 0.8	4.39 ± 1.4	5.35 ± 1.3	5.82 ± 1.4	6.27 ± 1.0
		transverse	1.98 ± 0.5	3.82 ± 1.2	4.83 ± 1.6	5.25 ± 1.5	5.76 ± 1.5	2.02 ± 0.3	3.95 ± 1.4	4.88 ± 1.6	5.67 ± 1.6	6.39 ± 1.4
	6 Hz	Longitudinal	2.46 ± 0.4	4.47 ± 1.4	4.88 ± 1.3	5.83 ± 1.0	6.25 ± 1.0	2.58 ± 0.9	3.90 ± 1.0	5.01 ± 1.5	5.81 ± 1.0	6.10 ± 0.9
		transverse	2.25 ± 0.4	3.47 ± 1.2	4.49 ± 1.6	5.43 ± 1.5	5.55 ± 1.5	2.04 ± 0.4	3.29 ± 1.1	4.33 ± 1.4	5.20 ± 1.5	5.72 ± 1.3
	12 Hz	Longitudinal	2.57 ± 0.6	4.37 ± 1.2	4.97 ± 1.2	5.63 ± 1.3	6.08 ± 0.7	2.29 ± 0.3	4.12 ± 1.2	4.61 ± 1.3	5.56 ± 1.1	6.14 ± 0.6
		transverse	2.16 ± 0.9	3.04 ± 1.1	4.55 ± 1.0	5.51 ± 1.4	6.27 ± 1.2	2.02 ± 0.6	3.53 ± 1.6	4.12 ± 1.5	5.10 ± 1.4	5.82 ± 1.0
	24 Hz	Longitudinal	2.57 ± 0.5	4.7 ± 1.6	5.19 ± 1.4	6.02 ± 1.6	6.12 ± 1.4	2.64 ± 0.9	4.12 ± 1.4*	4.86 ± 1.4	5.36 ± 1.3	5.95 ± 1.5
		transverse	2.14 ± 0.5	3.71 ± 1.1	4.73 ± 1.4	5.69 ± 1.5	5.81 ± 1.1	2.06 ± 0.3	3.65 ± 1.6	4.87 ± 1.8	5.51 ± 1.4	6.02 ± 1.0

MVC; maximum voluntary contraction, MG; medial gastrocnemius, SOL; soleus. *Significant changes between pre and post WBV ($p < 0.01$).

compared to 4.71 m/s (3.72–5.70 m/s) before WBV, as shown in Fig. 6D. An example of this change was shown in Fig. 6 after 0, 6, 12 and 24 Hz WBV in both image views.

3.2. Subjective muscle stiffness assessment

The subjective rating of calf muscle stiffness indicated greatest stiffness ($+1.6 \pm 0.7$ scale) after 12 Hz, followed by 6 & 24 Hz interventions ($+0.9 \pm 1.0$ & $+1.4 \pm 1.2$ scale, respectively) and after 0 Hz control (-0.4 ± 1.2 scale) (Table 3). The rating post 12 Hz WBV frequency was significantly higher than after 0 Hz (1.9 scale (1.2–2.8 scale), $p < 0.001$) and 6 Hz (0.7 scale (0.1–1.2 scale), $p = 0.031$).

3.3. Plantar flexors muscle architecture

From the longitudinal view of taken ultrasound images, the PA and FL for MG muscle and PA for SOL muscle were investigated as the results example shown in Fig. 7 after 0, 6, 12 and 24 Hz WBV interventions. Four-way repeated ANOVA yielded main effects of muscle ($F(1,11) = 94.8$, $p < 0.001$; $\eta^2 = 0.90$), time ($F(1,11) = 11.5$, $p = 0.006$; $\eta^2 = 0.51$), and contraction level ($F(4,44) = 254.5$, $p < 0.001$; $\eta^2 = 0.96$). PA changes were greater in MG muscle than SOL muscle, and all PA was increased monotonously along with the contraction level increased. Two-way repeated ANOVA demonstrated that PA for MG muscle was substantially decreased following 24 Hz intervention at 25 % and 75 % force level ($F(1,12) = 9.0$, $p = 0.011$; $\eta^2 = 0.43$, Fig. 7D), but this similar change did not happen for SOL and immediately after other WBV frequency. There were, however, no main effects of WBV frequency and intervention effect on FL changes ($p > 0.05$), but the FL of MG was

indeed shortened when the contraction level increased ($F(4,48) = 123.0$, $p < 0.001$; $\eta^2 = 0.91$).

3.4. Electromyography

As expected, all RMS amplitude values increased with the level of contraction, with greater RMS amplitudes for greater contraction levels ($F(3,18) = 74.6$, $p < 0.001$; $\eta^2 = 0.92$) as well as changed RMS was seen after the intervention ($F(1,6) = 8.8$, $p = 0.025$; $\eta^2 = 0.60$), regardless of vibration frequency ($F(3,18) = 0.66$, $p = 0.587$; $\eta^2 = 0.09$). This change was differently revealed among the 4 muscles ($F(3,18) = 18.7$, $p < 0.001$; $\eta^2 = 0.76$) when approached by four-way repeated measure ANOVA. The interaction effect between muscle RMS and contraction level was found ($F(9,54) = 16.6$, $p < 0.001$; $\eta^2 = 0.73$). Similarly, contraction level was also either affecting MF ($F(3,21) = 10.4$, $p < 0.001$; $\eta^2 = 0.60$) or among between the muscles for MF ($F(3,21) = 4.1$, $p = 0.02$; $\eta^2 = 0.37$) with regardless in the main effect of intervention ($F(1,7) = 0.83$, $p = 0.39$; $\eta^2 = 0.11$) and vibration frequency uses ($F(3,21) = 1.6$, $p = 0.22$; $\eta^2 = 0.19$).

By the two-way repeated approached, for the MG muscle a post 24 Hz intervention had an observed enhancement in percRMS amplitude at 50 % force level ($F(1,11) = 14.8$, $p = 0.003$; $\eta^2 = 0.57$) (Fig. 8H), Similar changes were seen for LG after 0 Hz intervention increasing RMS at MVC level ($F(1,11) = 15.1$, $p = 0.003$; $\eta^2 = 0.58$, Fig. 8E), for TA muscle following 6,12 & 24 Hz (all $p < 0.05$, $\eta^2 > 0.54$) and after every WBV intervention sessions for SOL muscle (all $p < 0.05$, $\eta^2 = 0.38$–0.68) as shown in Fig. 8E and H, an example following 0 and 24 Hz WBV intervention.

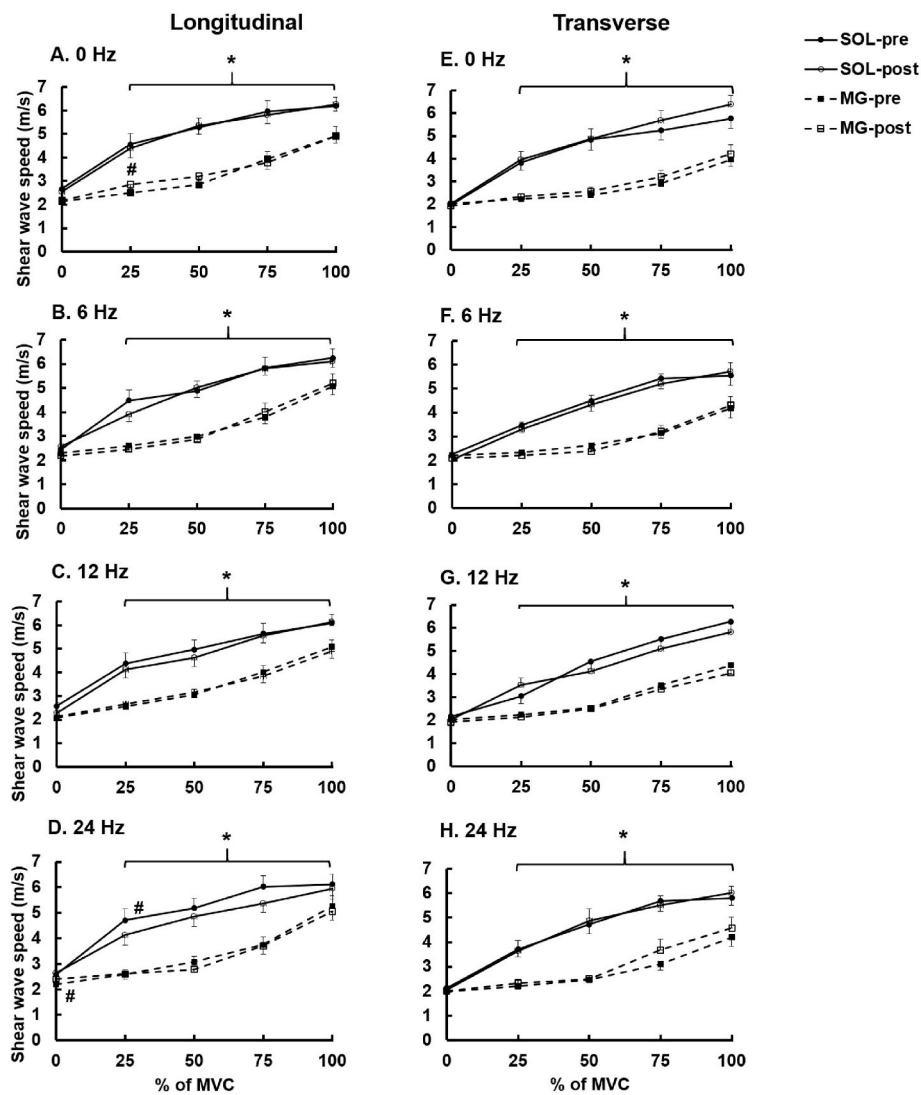For MF, there was no WBV intervention effect on MG, LG, and TA

Fig. 6. The change of shear wave speed after 0, 6, 12, 24 Hz WBV in MG and SOL. A-D. Longitudinal probe orientation to the muscle belly before & after 0, 6, 12, 24 Hz WBV. E-H. Transverse probe orientation to the muscle belly before & after 0, 6, 12, 24 Hz WBV. Dashed lines, represents MG and solid lines, SOL. Closed symbols, pre WBV and open symbols, post WBV. Data are mean and SEM. * Significant difference between MG and SOL. # Significant change between pre and post WBV intervention.

Table 3
Subjective calf muscle tone and change in resting shear wave speed after WBV of MG muscle.

WBV frequency	Subjective tone	SWS longitudinal		SWS transverse	
		Pre WBV	Post WBV	Pre WBV	Post WBV
0	−0.4 ± 1.2	2.14 ± 0.3	2.17 ± 0.3	2.02 ± 0.4	1.93 ± 0.2
6	+0.9 ± 1.0*	2.29 ± 0.7	2.18 ± 0.3	2.21 ± 0.4	2.09 ± 0.4
12	+1.6 ± 0.7*	2.14 ± 0.3	2.07 ± 0.4	2.41 ± 1.4	1.92 ± 0.3
24	+1.4 ± 1.2*	2.19 ± 0.3	2.40 ± 0.2#	1.97 ± 0.4	2.01 ± 0.3

WBV; whole-body vibration, MG; medial gastrocnemius. *Significant changes from control condition ($p < 0.05$). # Significant change between pre and post WBV intervention.

muscle whereas there was a greater change seen for SOL MF after 6 Hz intervention at 25 % contraction force ($F_{(1,9)} = 6.2$, $p = 0.034$; $\eta^2 = 0.41$).

3.5. Accuracy of vibration parameters

An overview of the vibration parameters per set frequency is given in Table 4. Small albeit significant ($p < 0.001$) deviations of the measured frequency from the set frequency were observed. Within trials, the vibration frequency was fairly constant, as evidenced by the coefficient of variation (CV) at or below 1 %. Peak-to-peak amplitude differed by a small amount between 6 Hz and the two higher frequencies ($p < 0.001$); however, this could be due to imprecisions in re-positioning the accelerometer between sessions. Finally, the mechanical vibration signal was almost perfectly sinusoidal, as evidenced by the fact that ~99 % of the signal power was found in the first harmonic of the power spectrum.
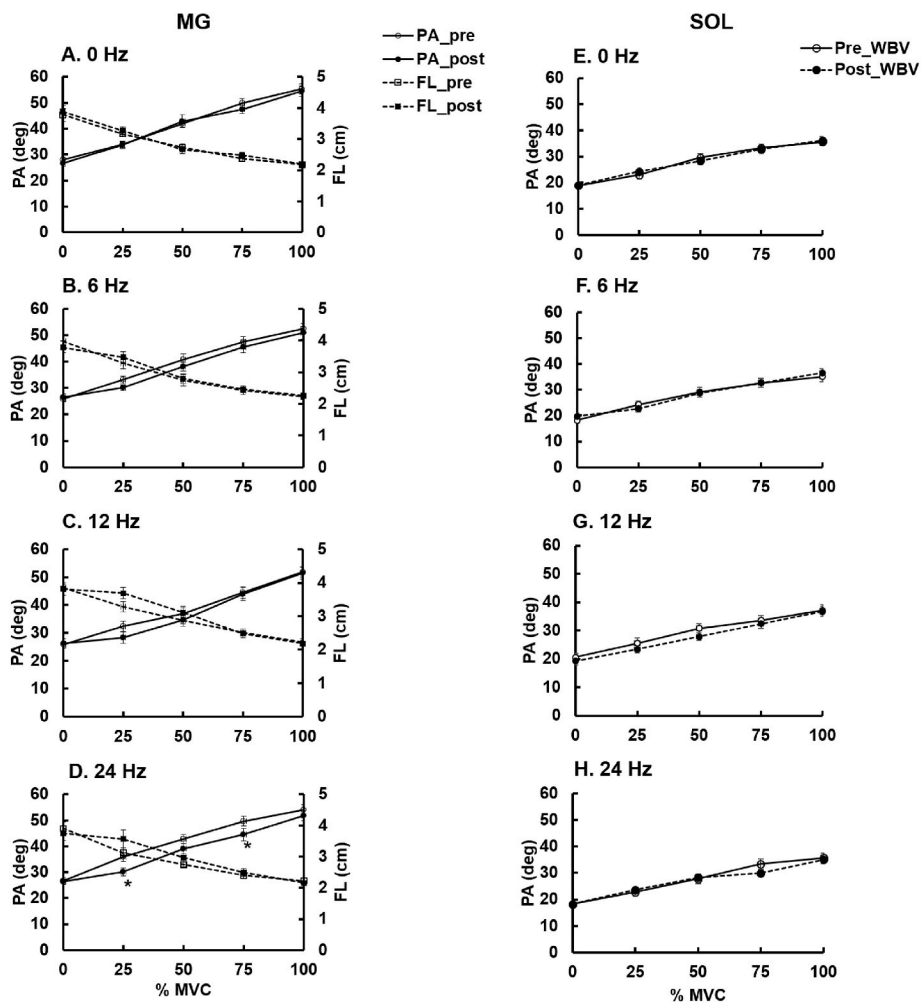
Fig. 7. The change of PF muscle architectures after 0, 6, 12, 24 Hz WBV. A-D. PA and FL of MG before & after 0, 6, 12, 24 Hz WBV.E-H. PA of SOL before & after 0, 6, 12, 24 Hz. Open symbols, represents pre WBV and closed one, post WBV. Circle symbols, PA and square symbols, FL. Data are mean and SEM. * Significant difference between pre and post WBV intervention.
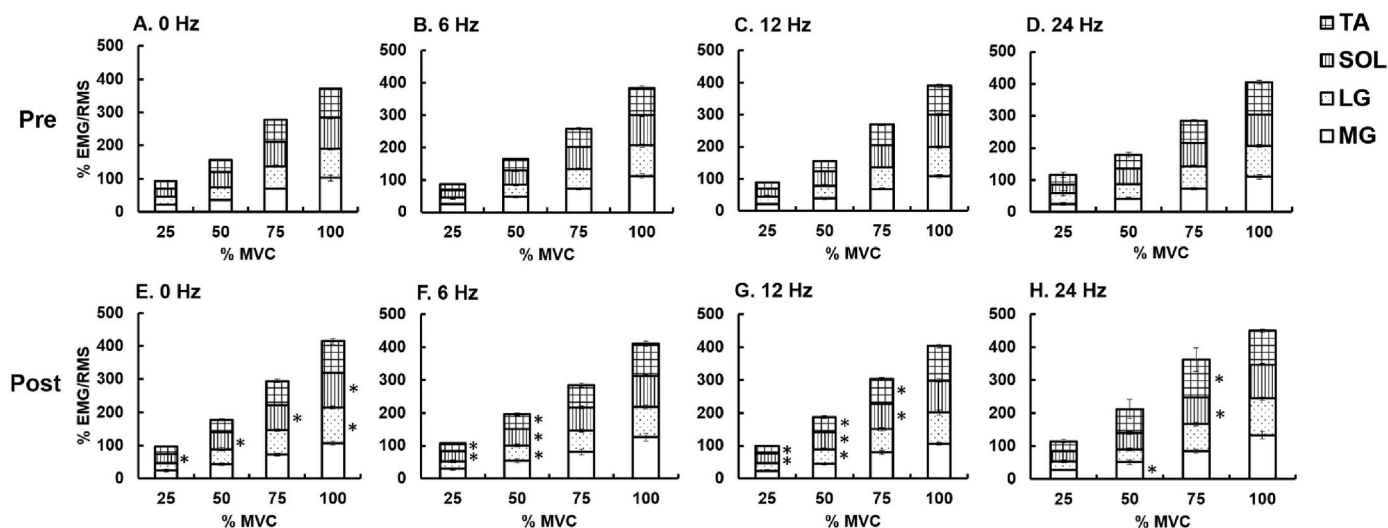

Fig. 8. The contribution of plantar flexors and antagonist muscle percRMS changes after 0, 6, 12, 24 Hz WBV. A-D. before 0, 6, 12, 24 Hz vibration. E-H. after 0, 6, 12, 24 Hz vibration. * Significant difference between pre and post WBV intervention.

Table 4
Mechanical vibration parameters, as measured via accelerometry.

	Set Frequency		
	6 Hz	12 Hz	24 Hz
Observed Frequency [Hz]	6.2 (0.02)	11.9 (0.04)	23.6 (0.04)
Frequency CV [%]	0.33 (0.04)	0.59 (0.12)	1.16 (0.02)
aRMS [g]	4.7 (0.03)	17.8 (0.15)	67.2 (1.75)
aMax [g]	7.00 (0.06)	24.91 (0.51)	97.86 (3.1)
Ap2p [mm]	9.36 (0.08)	8.86 (0.14)	8.93 (0.26)
Power 1ˢ Harmonic [%]	98.9 (0.13)	98.6 (0.38)	99.5 (0.11)

CV: coefficient of variation, assessed within trials and averaged across all trials; aRMS: averaged root-mean-square acceleration, given in multiples of 9.81 m/s^2 (g); aMax: peak acceleration within each cycle, given in g; Ap2p: peak-to-peak amplitude, given in mm; Power of the 1st harmonic in % of the total signal power. Data are given as means (SD).

4. Discussion

The starting point for this study were anecdotal reports of mechanical vibration induced-stiffness in healthy adults, with a purported dependency on vibration frequency. In apparent contrast with this hypothesis, the present study did not find any effect of vibration frequency at 6 Hz and 12 Hz on SWS at rest. However, we did find a significant increase in SWS in the longitudinal MG assessment following 24 Hz mechanical vibration, thus partly confirming our main hypothesis. By contrast, there was a moderate SWS reduction during contractions in longitudinal-projection in the SOL that was exclusively observed following 24 Hz mechanical vibration. This effect was paralleled by a reduction in MG PA, without any effect on FL. Subjectively, the greatest stiffness was reported after 12 Hz intervention, with effects being superior to 0 and 6 Hz while the resting MG SWS in longitudinal was higher after 24 Hz intervention. Lastly, plantar flexor peak isometric force was significantly higher following both mechanical vibration and 0 Hz sham interventions regardless of the changes in MG and SOL SWS and muscle architectures. In summary, SWS effects were limited to 24 Hz mechanical vibration and to the longitudinal projection.

Whilst the majority of participants subjectively felt most increased calf stiffness following 12 and 24 Hz WBV intervention, corresponding to the previous study that revealed the mechanical vibration frequency-dependence on increasing rate of discomfort on lower limbs,[17,43] the resting SWS after 12 Hz WBV was generally unaffected at rest, with the exception being a decreased longitudinal SWS in the MG. This suggests that subjective feeling was unrelated, or even somewhat opposed to the resting elastic properties inside the targeted muscle. It is currently unclear how the subjective feeling of muscle stiffness emerges, but Golgi tendon organ and muscle spindles are likely involved, as well as the spinal neuronal circuitry. One has to consider that the spindles and Golgi tendon organ are located towards the myotendinous junction,[44] whilst the elastography assessment in this study was performed within the 'working' muscle and thus excluded the myotendinous junction. It thus becomes clear that concordance of subjective ratings with objective measures of stiffness may not necessarily be expected, in particular when considering alterations in the fascicles' contractile state. More precisely, in a Hill-type muscle model,[45] enhanced contractile element tension is bound, for the same series-element tension, to reduce the parallel-elastic tension thus offering a potential explanation for the seemingly contradictory findings in subjective and objective stiffness changes at rest. Hence, the concordance of increasing subjective ratings and muscle stiffness following 24 Hz intervention questions the perceptual ability of subjects to distinguish between these two high-intensity WBV.

Of interest in this study is also the SWS reduction in the SOL muscle at 25% contraction following 24 Hz vibration. Whilst this could be simply unrelated to the increased SWS observed in the MG muscle, it has to be considered that SOL, LG and MG are linked in their mechanical output via the Achilles tendon. Because of this arrangement, those three

muscle heads of the triceps surae complex add their force upward to generate the reactive force of the Achilles tendon.[46] Accordingly, when the contractile state in one of these muscles increases, then the mechanical contribution of the others will, for the same Achilles tendon reactive force, decrease. Hence, in continuation of the above-proposed interpretation of an enhanced MG muscle contractile state, reduced stiffness in SOL muscle could be well explained. We have also observed reduced PA in MG at 25 and 75% contraction levels following specifically 24 Hz vibration. This would likewise be explained by reduced force contribution of the MG muscle (Fig. 7), and it also suggests, in combination with our finding of generally greater SWS in longitudinal than in transverse projection, that the observed SWS effects in SOL were not caused by alterations in PA (Fig. 7D) – rather, based on the fact that SWS is generally greater in longitudinal than in perpendicular fascicle projection, one would expect a reduced PA to result in increased SWS in longitudinal projection, and not in reduced SWS as found in the previous study,[47] which found a similar change during PF contraction in the knee-flexed position.

The application of SWS assessment in skeletal muscle is relatively new. Previous studies have documented that SWS increases with muscle-tendon complex length (i.e. passive tension),[48] likely via affecting pre-tension of the resting muscle. Moreover, SWS also increases with muscle force in longitudinal[33] but not in transverse projection.[49] Our study, confirms contraction-dependence in longitudinal projection, and it extends this finding also to the transverse projection (Fig. 6). In addition, we have demonstrated that SWS is larger in SOL than in MG muscle.

Relatively little is known about acute effects of physical interventions on SWS. Two recent studies report reduced muscle stiffness following acute static stretching,[50,51] one of which confirmed the SWE-based measurements with traditional ultrasound-based length-force relationship. In consideration that vibration exercise does not acutely affect stiffness of the series-elastic element,[26] and thus likely also not the parallel-elastic element, this gives further support to an alteration of the fascicle's contractile state[14] for an explanation of the increased SWS in resting MG following 24 Hz in this study. In addition, Bernabei et al.[52] have studied the effects of temperature on SWS in contracting muscle in anesthetized cats. The authors report a decrease in SWS by 1 m/s at a given force for a rise in temperature from 26% to 38%. Moreover, SWS decreases in quadriceps muscle have been demonstrated acutely after finishing an ultra-marathon,[53] a sport event that increases muscle temperature. Given the known effects of mechanical vibration on muscle temperature,[54] that offers a viable explanation for the SWS reduction in SOL muscle following 24 Hz vibration.[52]

The question also arises why previous studies have been unable to find WBV effects on muscle stiffness.[25,30,31] All of these studies have used different technical approaches, such as damped-oscillation,[25] ultrasound-indentation,[30] or hopping-based assessment of stiffness.[31] We argue that the SWE approach in the present study is able to differentiate between specific muscles (as opposed to whole-limb effects), and also between the longitudinal (muscle's axis of pull) and transverse axes. This study therefore was able to monitor the mechanical vibration with greater fidelity as the aforementioned studies, which seems to validate the use of SWE in muscle exercise and rehabilitation studies. Moreover, the use of WBV at high frequency as pre-therapy or warm up before specific physiotherapy or occupational therapy is recommended to facilitate muscle temperature[54] and muscle activity.

4.1. Limitations

The present study has several limitations. First of all, muscle strength in the baseline measurements increased with each visit (Table 1, lower part). This is a well-known weakness of muscle strength measures. Luckily, muscle strength was not an endpoint of this study. Moreover, the sequential assignment to the intervention conditions was balanced, so that plantar flexor strength was comparable across condition (Table 1,

upper part). Second, we saw an increase in plantar flexor strength between baseline and post-intervention within each visit. This effect was rather unexpected. Hence, this MVC change seems to be unrelated to mechanical vibration exposure, but more likely is due to maintaining the squat position at 20° knee flexion with heel slightly raised during the intervention, or to a 'potentiation' effect by the MVC testing itself. Of note, the so-called post-activation potentiation phenomenon is well-established.[55] To further understand the effect and its implications, we propose that future studies with comparable design include a 'pure' control conditions with endpoint measurements only, and without any intervention altogether. With regards to the present study, we feel that its result stand, given that the effect on MG muscle was limited to resting conditions, and that the effects upon SOL consisted in reduced SWS, and not in increased SWS as would be expected from increased contraction levels. Third, the interpretation of results is hampered by the difficulty to link changes in muscle's material stiffness (in two directions) with changes in PA and plantar flexor contraction force from the 3 different constituents of the triceps surae muscle. Such a model is lacking in literature, and the explanation proposed here can only be regarded as a first attempt.

4.2. Strengths of the study

This research employed a controlled, cross-over (balanced) design, offering the advantage of within-subject comparisons that mitigate variability. The potential utility of SWE becomes substantial evidence when assessing the soft tissue stiffness response to interventions or exercise training of this nature.

5. Conclusion

The 5-min mechanical vibration intervention acutely elevates SWS in the MG muscle at rest and in the SOL muscle during contraction, accompanied by alterations in MG PA only following 24 Hz WBV. Potential underlying physiological mechanisms contributing to these effects include post-activation potentiation, warm-up, and force distribution within the triceps surae muscle. It is unlikely that these effects stem from modifications in the parallel-elastic or series-elastic elements. Interestingly, self-reported sensations of stiffness were noted at 12 Hz but not at 24 Hz vibration frequency. This discrepancy may be attributed to the divergent anatomical sites of physiological sensors and the measurement of SWS.

Author contributions

Conceptualization, J.R., C.H. and S.K.; methodology, P.M. , C.H.; formal analysis, P.M., J.R., U.M., J.Z.; investigation, P.M., C.H., W.S; data curation, P.M., U.M.; writing—original draft preparation, J.R., C. H., P.M., E.S.; writing—review and editing, P.M., J.R., C.H., I.D.; supervision, J.R.; project administration, J.R.; funding acquisition, J.R. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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