TITLE:

Whole body vibration of different frequencies inhibits H-reflex but does not affect voluntary activation.

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ABSTRACT

This study aimed to investigate the effects of whole-body vibration (WBV) at a frequency spectrum from 20 to 50 Hz on the Hoffmann (H) reflex and the voluntary motor output of ankle plantar-flexor muscles. A single-group (n: 8), repeated measures design was adopted with four conditions: standing (no vibration), 20, 35 and 50 Hz, each lasting one minute. H-reflex of the soleus muscle, maximal voluntary contraction (MVC) and central activation ratio (CAR) of the plantar-flexors were evaluated before, 1 and 5 min after each frequency condition. H-reflex decreased by 36.7% at 20 Hz, by 28% at 35 Hz, and by 34.8% at 50 Hz after one minute from WBV compared to baseline. Neither MVC nor CAR changed after WBV at all frequency conditions. The short-term, acute inhibition of the H-reflex after WBV at 20, 35 and 50 Hz suggested that decreased excitability of spinal motoneurons is not frequency dependent. On the other hand, the lack of vibration induced effects on MVC and CAR indicated that a 1-min WBV stimulus is not sufficient to affect the voluntary motor output.

1. INTRODUCTION

Acute exposure to whole-body vibration (WBV) has been reported to cause short-term improvements of lower limb muscle performance in a number of lower limb motor tasks (Bosco, Cardinale, & Tsarpela, 1999; Cormie, Deane, Triplett, & McBride, 2006; Giombini et al., 2013), however the underlying neural mechanisms are still under debate (Sayenko, Masani, Alizadeh-Meghrazi, Popovic, & Craven, 2010; Cochrane, 2011; Giombini et al., 2015). Oscillatory mechanical stimuli delivered to the lower limbs via the feet during WBV are thought to elicit rapid lengthening/shortening of the lower limb muscles, thereby activating the muscle spindle Ia afferents and evoking muscle contractions via the stretch reflex pathway (Ritzmann, Kramer, Gruber, Gollhofer, & Taube, 2010); in turn, this is believed to increase sensitivity of the muscle spindles and facilitate the subsequent efferent output (Cardinale & Bosco, 2003; Rittweger, 2010). On the other hand, a strong discharge in Ia afferents during local vibration does result in suppression of the spinal excitability of stretch reflex, as investigated by the Hoffmann (H) reflex (McNeil et al., 2013), probably via presynaptic inhibition (Arcangel et al. 1971; van Boxel 1986). Previous studies on spinal modulation of the stretch reflex after termination of whole-body vibration have reported equivocal findings including unchanged (McBride et al., 2010), decreased (Sayenko et al., 2010; Armstrong et al., 2008; Kipp, Johnson, Doeringer, & Hoffman, 2011; Games & Sefton, 2013; Ritzmann, Gollhofer, & Kramer, 2013; Krause, Gollhofer, Freyler, Jablonka, & Ritzmann, 2016), and increased H-responses immediately following WBV exposure (Nishihira, Iwasaki, Hatta, & Wasaka…, 2002). (Hortobagyi, Rider, & DeVita, 2014) reported that WBV at 30 and 50 Hz induced initial depression of the soleus H-reflex responses followed by a facilitation, although there was no change in the volitional wave and the H-reflex during ongoing muscle contractions, indicating no acute effects of WBV on the efferent neural drive to the muscle and on spinal excitability, respectively. Recently, a detailed study by Harwood et al. (2017) reported

64 a 46% reduction in the soleus H-reflex amplitude after five 1-min sets of WBV at 45 Hz, with
65 concomitant decrease in peak twitch torque and rate of twitch torque development (i.e.
66 contractile function), while percent voluntary activation and maximal plantar flexor torque were
67 unchanged as a consequence of WBV.

68 The neuromuscular response to WBV has been shown to depend on factors such as the
69 type, frequency, amplitude and duration of the oscillatory stimulus (Di Giminiani, Masedu,
70 Tihanyi, Scrimaglio, & Valenti, 2013; Krause et al., 2016; Pistone et al., 2016). Among all of
71 these factors, it is the frequency of WBV that has received increased attention due to its well-
72 known relationship with the magnitude of activation in the lower limb muscles during vibration;
73 for instance, a gradual rise in WBV induced muscle activity has been observed up to frequencies
74 of 30-35 Hz (Ritzmann et al., 2013; Pollock, Woledge, Mills, Martin, & Newham, 2010),
75 followed by a decrease in muscle activity as WBV frequency increases (Carlucci et al., 2015;
76 Di Giminiani et al., 2013; Giombini et al., 2015). Interestingly, greater improvements in motor
77 performance of the lower limb muscles have been reported after applying WBV at frequencies
78 of 25-35 Hz compared to either lower or higher vibratory frequencies (Giombini et al., 2013).
79 Therefore, it could be hypothesised that the reflex inhibitory inflow acting at spinal level might
80 become less predominant and lead to increased voluntary activation after applying WBV
81 stimuli at "optimal" frequencies of about 30 Hz compared to lower and higher frequencies. To
82 the best of the authors' knowledge, however, the stretch reflex excitability responses and the
83 change in motor output have never been assessed jointly in one study focusing on WBV at
84 different oscillatory frequencies.

85 Therefore, the purpose of the present study was to investigate the acute effects of WBV
86 over a frequency spectrum from 20 to 50 Hz on the soleus H-reflex excitability and the major
87 determinants of motor output of the ankle plantar-flexor muscles in healthy young individuals.
88 As greater improvements in motor performance were reported after applying WBV at

frequencies of 25-35 Hz compared to either lower or higher frequencies (Giombini et al., 2013), it was hypothesised that a WBV stimulus at 35 Hz would be associated with lower inhibition of the H-reflex responses and higher increase in force output as compared to WBV stimuli at 20 and 50 Hz.

2. MATERIALS AND METHODS

2.1 Participants

Eight young males (age: 26 ± 4 years, height: 1.74 ± 0.02 m, body mass: 74.6 ± 5.1 kg), with no history of neurological or orthopaedic disorders, volunteered to participate in the study. Only physically active individuals who were not engaged in regular training or sport practice more than three times a week, for more than 40–60 min each time, were included in the study. None of the participants had experience with WBV exercise prior to experimental sessions. This study was approved by the local Ethics Committee and carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

2.2 Study design

A single-group, repeated measures, crossover study design was adopted with four WBV frequency conditions: standing (no vibration), 20, 35 and 50 Hz. Each frequency condition lasted 1 min, while all participants maintained a similar posture on the platform. For each frequency condition, a set of reflex and motor measures were initially undertaken as baseline values, and were then repeated 1 and 5 min after the WBV exposure. The order of frequency conditions was randomised across participants and it was allowed a 30-min recovery period between the end of one frequency condition measure and the beginning of the following one. Experimental sessions were conducted at identical time of the day and environmental conditions.

2.3 Whole body vibration set-up

The participants were exposed to synchronous vertical oscillations at 4-mm peak-to-peak amplitude using a WBV platform (NEMES-LC; BoscoSystem Technologies, Rieti, Italy). Each subject stood barefoot on the platform to eliminate any damping of mechanical oscillations that could be due to footwear. During the exposure to WBV, participants were asked to stand on the forefoot with an angle of 90° at the ankle joint and 10° at the knee joint (0° corresponding to the knee full extension), and to distribute their weight evenly on both sides with the feet positioned 2 cm apart (measured between medial malleoli). A foam cube (3 × 3 × 3 cm) was placed under the participant's heels in order to control the forefoot stance and to reduce the variance in ankle joint position within and between subjects (Ritzmann et al., 2013) by asking the participants to keep contact with the cube without deforming it throughout the WBV exposure. Angular displacement of the ankle joint was controlled by means of an electrogoniometer (Biometrics Ltd., Gwent, UK OR Penny & Giles, Santa Monica, CA), which was placed on the lateral side of the dominant limb with the two arms aligning to the leg and foot axes. The knee joint angle was checked with a goniometer prior to administration of WBV. Throughout each WBV treatment, the investigators made sure that the participants' trunk did not lean laterally, the knee angle was held constant and the heels were not raised from the cube.

2.4 H-reflex stimulation and recording procedures

During the test, participants semi-reclined comfortably on a dynamometer chair (Kin Com, Chattanooga, TN), with their trunk fastened by three crossing belts. The seatback tilt of the chair was set to maintain a hip joint angle of 90°, a knee joint angle of 10° and an ankle joint angle of 90°. The foot was secured to the dynamometer pedal via straps placed over the metatarsal, ankle and heel regions to eliminate heel movement. Standard procedures for testing

139 the soleus H-reflex in the dominant limb were followed as previously described by others
140 (Hugon, 1973; (Zehr, 2002). The posterior tibial nerve was electrically stimulated via surface
141 electrodes with a constant voltage isolated stimulator (Digitimer, model DS7A; shocks of 1 ms
142 duration) with the cathode (8 mm in diameter) located in the popliteal fossa and the anode
143 placed on the anterior aspect of the right knee just above the patella. Both cathode and anode
144 were surrounded by an elastic rubber strap to maintain a constant pressure on the electrodes and
145 to ensure minimal displacements during the protocol. The interval in between stimulations was
146 10 s, in order to rule out the effects of post-activation depression on the reflex responses.[24] To
147 obtain a complete recruitment curve of the H-reflex, the electrical stimulus intensity was
148 increased at steps of 2 mA until the maximal motor direct response (M-max) was obtained.
149 Electromyography (EMG) responses of the soleus muscle to the nerve stimulation were
150 recorded with bipolar surface electrodes (LISiN, Turin, Italy; 10 mm of inter-electrode
151 distance) placed on the dominant limb 2–3 cm below the point where the two heads of
152 gastrocnemius muscle join in the Achilles' tendon, and parallel to the muscle fibres. A ground
153 electrode (silver plate; 2 × 3 cm) was placed over the lateral malleolus of the fibula in the
154 ipsilateral limb. Before applying the adhesive surface electrodes, the skin was shaved and gently
155 abraded with abrasive paste (Meditec-Every, Parma, Italy). Medical adhesive tape and an elastic
156 band were then used to fix the EMG cables to the skin in order to minimize any motion artefacts
157 that could be encountered during the vibration. The EMG signal was amplified (×2000), band-
158 pass filtered (10 Hz - 500 Hz) and sampled at 2048 Hz using a multichannel bioelectrical signal
159 amplifier (EMG-USB2, OT Biolelettronica, Turin, Italy). Peak-to-peak EMG amplitude of both
160 reflex and motor responses of the soleus muscle were evaluated using the OT biolab software
161 (OT Biolelettronica, Turin, Italy) and then used for further analysis.

162 For each WBV frequency condition, H-reflex test recordings started at least 30 s
163 following the end of vibration exposure to avoid post-contraction depression of the reflex

responses (Schieppati & Crenna, 1984). The test reflex stimulus intensity was selected to obtain an H-reflex on the ascending limb of the curve with peak-to-peak amplitude of about 1/2 of H-max, so that a small amplitude M-wave was also evoked and monitored as a means of estimating and controlling stimulus consistency (Zehr, 2002; Laudani, Wood, Casabona, Giuffrida, & De Vito, 2009). Only H-reflex tests which showed an M-wave with an amplitude within the range of ± 5% of control values were accepted (Fig. 1). After achieving a minimum of three acceptable H-reflex tests, with a 10-s interval between stimulations, three M-max responses were also collected before, 1 and 5 min after each frequency condition (standing, 20, 35 and 50 Hz). All H-reflex amplitudes were normalised to the M-max amplitude and expressed as percentages. Values of each set of three H-reflex and M-max normalised amplitudes were averaged off-line and used for further analysis.

2.5 Motor output measurement

Isometric maximal voluntary contraction (MVC) of the plantar-flexor muscles was evaluated in the dominant lower limb by a dynamometer (Kin Com, Chattanooga, TN) immediately after the end of each H-reflex recordings set. Throughout MVC testing, participants maintained the semi-reclined body posture adopted during the H-reflex recordings, with the talus and medial malleolus (i.e. the axis of rotation of the ankle joint) aligned with the centre of rotation of the dynamometer shaft. The MVC task consisted of a quick increase to a maximum in the force exerted by the plantar flexors. A target line was always set on the computer screen at a value 20% higher than the best performance (Laudani et al., 2013). Participants were able to follow their performance on the computer screen and were verbally encouraged to achieve a maximum and to maintain it for at least 3 s before relaxing. MVC was calculated as the largest 1-s average reached within any single force recording. A minimum of three attempts was performed separated by 3 min, and that with the highest force value was

chosen as MVC. Participants were asked to make a further attempt if the MVC of their last trial exceeded that of previous trials.

Superimposed to each MVC trial, a supramaximal current stimulus was delivered to the posterior tibial nerve at a 150% intensity of that associated to the maximal M-wave response immediately after the peak torque. The rate of voluntary activation was then calculated according to the Central Activation Ratio (CAR) method described in Krishnan & Williams (2010) as follows: CAR (%) = voluntary torque at the time of stimulus delivery to the peak force / torque measured during superimposition of electrical pulses × 100.

2.6 Data analysis and statistics

Statistical differences in H-reflex measures (normalised H-reflex, small M-wave and M-max) and motor output measures (MVC and CAR) were evaluated by a two-way repeated measures analysis of variance (ANOVA) with frequency condition (standing, 20, 35 and 50 Hz) and time (pre, post 1 and 5 min) as within-subjects factors. Data were checked for normality and sphericity by the Mauchly test and, when a significant frequency × time interaction was found, a repeated-measures ANOVA was used to evaluate the significant differences between different times within each frequency condition. Post-hoc pair-wise comparisons were performed with Bonferroni-corrected paired t-tests. Data analysis and statistics were performed using a statistical package software (SPSS version 20.0, Inc., Chicago, IL-IBM, Somers, NY, USA). The level of significance was set to $P < 0.05$.

3. RESULTS

3.1 H-reflex modulation

The ANOVA showed no significant difference in the amplitude of M-max across WBV Frequency and Time conditions (Tab 1; $P > 0.05$).

There was a main effect of Time ($F = 11.21$, $P < 0.01$) and a Frequency × Time interaction ($F = 3.22$, $P < 0.05$) on the H-reflex amplitude normalised to the M-max, with no concomitant difference in the small M-wave associated to the test H-reflex. Follow-up analysis revealed no significant effect of Time on the normalised H-reflex amplitude at WBV frequency of 0 Hz, while a significant effect of Time on the normalised H-reflex amplitude was found at WBV frequency of 20 Hz ($F = 9.58$, $P < 0.01$), 35 Hz ($F = 4.04$, $P < 0.05$), and 50 Hz ($F = 12.78$, $P < 0.01$). At WBV of 20 Hz, the normalised H-reflex amplitude decreased on average by 36.7% one minute after WBV significantly relative to baseline values; at WBV of 35 Hz, the normalised H-reflex amplitude one minute after WBV significantly decreased on average by 28% relative to baseline values; at WBV of 50 Hz, the normalised H-reflex amplitude one minute after WBV significantly decreased on average by 34.8% relative to baseline values. Amplitude of H-reflex responses returned to baseline values five minutes following WBV at all frequency conditions.

3.2 Motor output measures

As reported in tables 2 and 3, the ANOVA did not show any significant main effect or interaction for MVC ($P > 0.05$) and CAR ($P > 0.05$), respectively.

4. DISCUSSION

The main finding of the present study was that WBV at 20, 35 and 50 Hz induced an acute inhibition of the soleus H-reflex amplitude as early as one minute after vibration exposure. On the other hand, there was no effect of WBV at any frequency on the MVC and CAR of the ankle plantar-flexor muscles, thus partially rejecting our hypothesis.

The acute decrease of the H-reflex responses one minute following WBV is in line with reports of previous studies on both local vibration (De Gail, Lance, & Neilson, 1966) and WBV

(Sayenko et al., 2010; Armstrong et al., 2008; Kipp et al., 2011; Games & Sefton, 2013; Ritzmann et al., 2013). As suggested by Armstrong et al (2008), the acute depression of the H-reflex after WBV might be due to muscle fatigue, which is known to increase pre-synaptic inhibition on Ia afferent terminals through stimulation of Golgi tendon organs and cutaneous mechanoreceptors via group III and IV afferents (Duchateau & Hainaut, 1993). However, in the present study, the lack of post-vibratory changes in the major determinants of the ankle plantar-flexors' motor output indicated that muscle fatigue did not occur at any of the testing times and/or vibration frequencies. One plausible mechanism could be suppression of muscle spindle activity, which has been reported to occur immediately after cessation of a 30-sec only exposure to muscle tendon vibration (Ribot-Ciscar et al. 1998). As suggested by (Sayenko et al., 2010), who reported a similar H reflex depression after exposure to one minute of vertical WBV at 35Hz, other mechanisms that might contribute to the reflex depression induced by short bursts of WBV might include pre-synaptic inhibition of Ia terminals with primary afferent depolarization and post-activation depression due to repetitive activation of the Ia-motoneuron synapse followed by reduced probability of transmitter release (Pierrot-Deseilligny & Mazevet, 2000). Since the H-reflex bypasses muscle spindles, and no changes in M-max amplitudes occurred after the vibratory exercise, it is logical to think that such depression resulted from mechanisms acting at spinal level on the synapse between the Ia afferents and the alpha motoneurones serving the plantar-flexor muscles. A complete recovery of the H-reflex response amplitude was observed after five minutes from WBV in this study, which is in line with previous studies adopting similar duration (i.e. one minute) of vibratory stimuli (Sayenko et al., 2010; Armstrong et al., 2008). On the other hand, previous studies using longer duration and higher amplitudes of vibratory stimuli compared to the present study have reported a reflex inhibition of longer durations; for instance, Games & Sefton (2013) showed that five one-

minute exposures of WBV at a frequency of 50 Hz caused H-reflexes to decrease for 20 minutes following the end of the vibration.

Exposure to WBV stimuli at all oscillatory frequencies in the study did not lead to significant change in the motor output of the plantar-flexor muscles. Previous studies have reported diverging findings with WBV leading to increase (Torvinen et al., 2002), decrease (de Ruiter et al., 2003) or having no effect (Jordan, Norris, Smith, & Herzog, 2010) on the lower limb muscles' MVC. Such equivocal findings might be due to the discrepancies across studies in the neuromuscular loading induced by WBV exercise protocol, which is a combination of type, frequency, amplitude and duration of the oscillatory stimulus as well as body position and external loading. For instance, Maffiuletti, Saugy, Cardinale, Micallef, & Place (2013) found no effect of 5-min WBV on MVC of the ankle plantar flexor muscles, despite the participants being required to hold on their shoulders an extra load of 50% of their body weight while maintaining an 80º knee angle position, likely due to the fact that such body position is mainly activating the quadriceps muscle group (Schoenfeld, 2010). Since the body position adopted in the present study is known to activate specifically the plantar-flexor muscles during WBV (Ritzmann et al., 2013; Di Giminiani et al., 2013), it is plausible to deduct that a 1 min duration of WBV exposure might have not been sufficient to induce significant changes in the muscle output following vibration. With this regard, future studies are warranted to investigate the acute effects of WBV stimuli of longer durations while maintaining body positions involving primarily the plantar-flexor muscles.

In conclusion, the present study results suggested that WBV at 20, 35, and 50 Hz affected the soleus H-reflex excitability one minute after vibration, causing depression of H-reflex responses. On the other hand, WBV at all frequencies had no effect on major determinants of force output, as indicated by the lack of post-vibratory change in MVC and CAR of plantar-flexor muscles. Future research should explore the effects of vibration

frequency applied for longer duration and/or higher amplitudes (e.g., longer protocols) than those adopted in the present study in order to gain a comprehensive understanding of the relationship between WBV frequency and the neuromuscular modulation of the plantar-flexor muscles.

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421

TABLES

Table 1. Maximal M-waves (M-max) of the soleus muscle before (PRE) and after one (POST-1) and five minutes (POST-5) following a 1-min WBV exposure at different vibratory frequencies. Data are presented as group means ± standard deviation.

M-max (mV)	**Standing**	**20 Hz**	**35 Hz**	**50 Hz**
PRE	3.23 ± 1.73	3.53 ± 1.08	3.31 ± 1.64	3.31 ± 1.71
POST-1	3.22 ± 1.69	3.47 ± 0.92	3.50 ± 0.96	3.51 ± 0.93
POST-5	3.26 ± 1.67	3.42 ± 0.81	3.36 ± 0.84	3.46 ± 0.89

Table 2. Torque of the ankle plantar-flexor muscles during a maximal voluntary contraction (MVC) before (PRE) and after one (POST-1) and five minutes (POST-5) following a 1-min WBV exposure at different vibratory frequencies. Data are presented as group means ± standard deviation.

MVC (Nm)	**Standing**	**20 Hz**	**35 Hz**	**50 Hz**
PRE	409.7 ± 189.5	363.9 ± 174.2	376.1 ± 152.5	359.4 ± 133.4
POST-1	376.1 ± 152.5	398.7 ± 188.3	408.8 ± 203.9	416.1 ± 184.7
POST-5	362.5 ± 168.0	417.9 ± 221.5	415.3 ± 204.3	422.5 ± 229.1

Table 3. Central activation ratio (CAR) of the ankle plantar-flexor muscles before (PRE) and after one (POST-1) and five minutes (POST-5) following a 1-min WBV exposure at different vibratory frequencies. Data are presented as group means ± standard deviation.

CAR (%)	Standing	20 Hz	35 Hz	50 Hz
PRE	91.2 ± 5.1	88.9 ± 8.6	90.1 ± 7.2	89.5 ± 9.3
POST-1	90.1 ± 7.2	89.9 ± 7.3	88.5 ± 8.4	90.4 ± 7.2
POST-5	88.5 ± 8.9	89.9 ± 9.1	90.1 ± 7.6	89.6 ± 8.0

FIGURE CAPTIONS

Figure 1. Test H-reflex and associated small M-wave. Only H-reflex tests preceded by M-wave with a peak-to-peak amplitude within the range of ± 5% of control values were accepted as a means of estimating and controlling stimulus consistency through experimental conditions.

Figure 2. Amplitude of the soleus H-reflex and associated M-waves normalised to M-max before (PRE) and after one (POST-1) and five minutes (POST-5) following a 1-min WBV exposure at standing condition with no vibration (a) and at a frequency of 20 (b) 35 (c) and 50 Hz (d). Data are presented as group means ± standard deviation. * = significantly different than PRE.

461 **FIGURE 1**

462

463

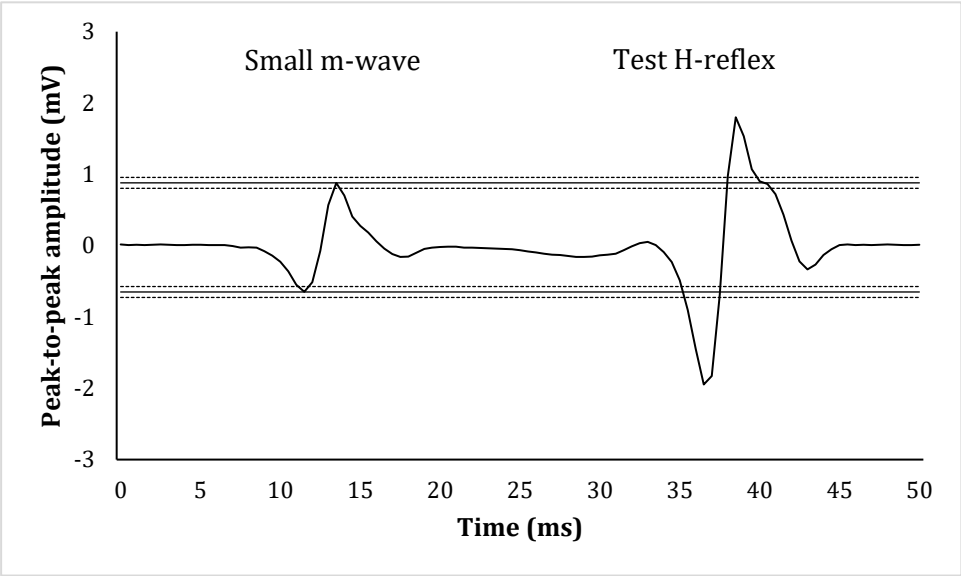


FIGURE 2A

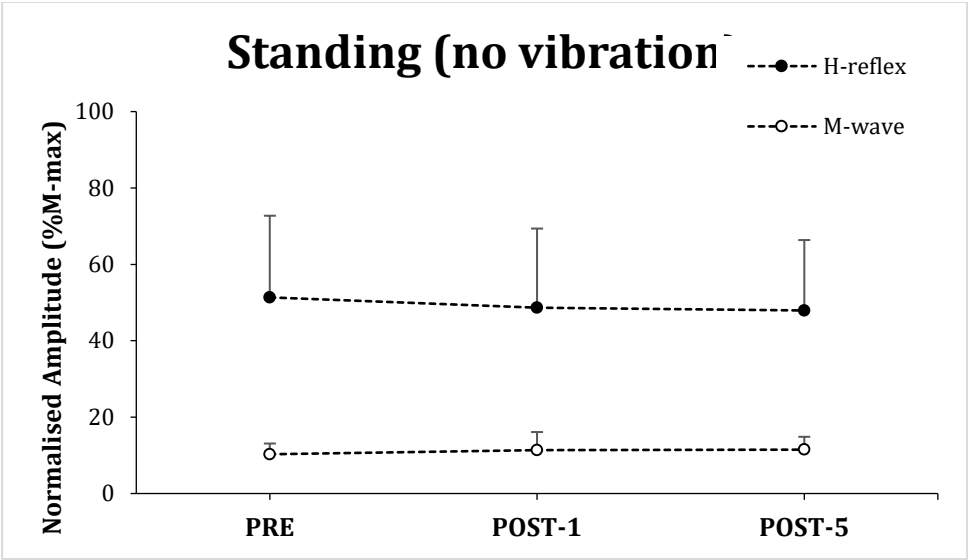

Standing (no vibration
H-reflex
M-wave
Normalised Amplitude (%M-max)
100
80
60
40
20
0
PRE
POST-1
POST-5

FIGURE 2B

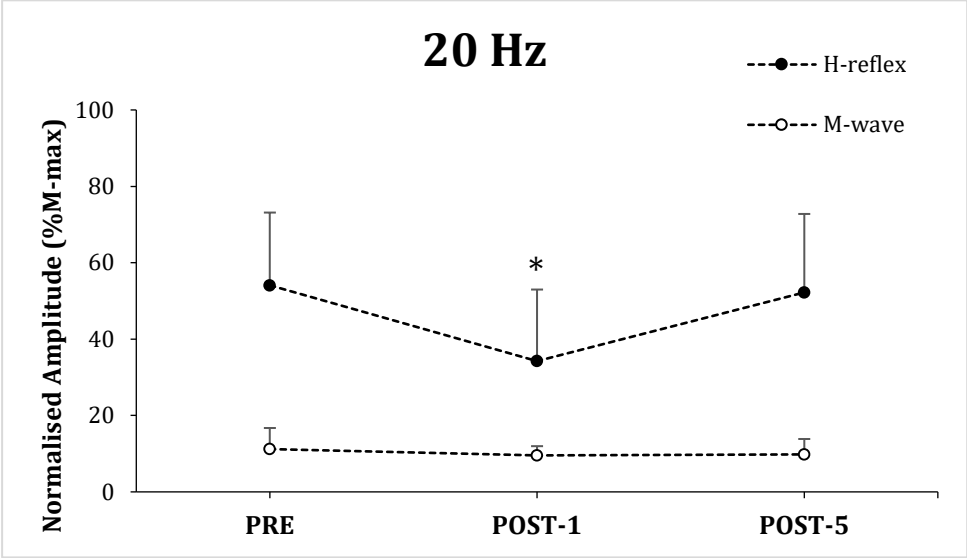

20 Hz
H-reflex
M-wave
Normalised Amplitude (%M-max)
100
80
60
40
20
0
*
PRE
POST-1
POST-5

FIGURE 2C

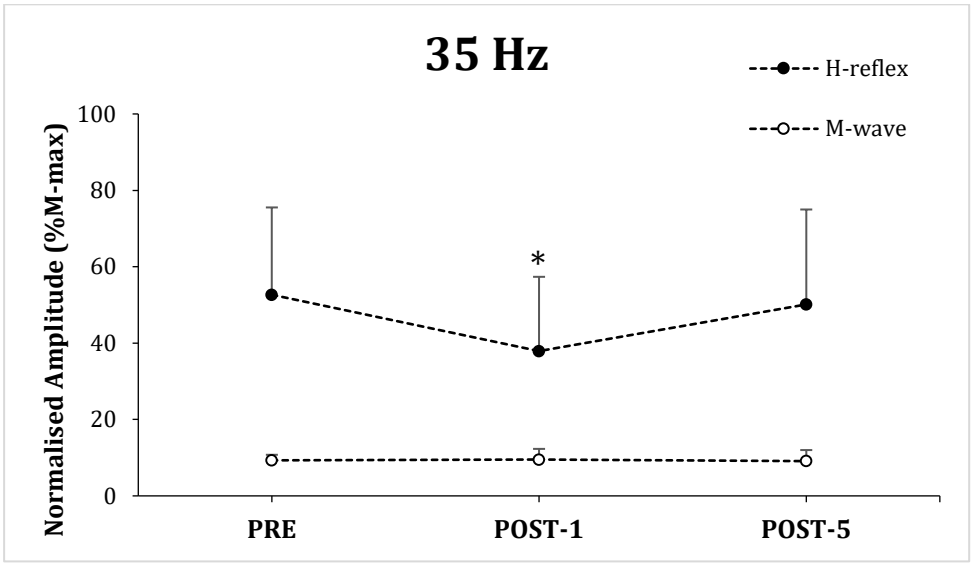
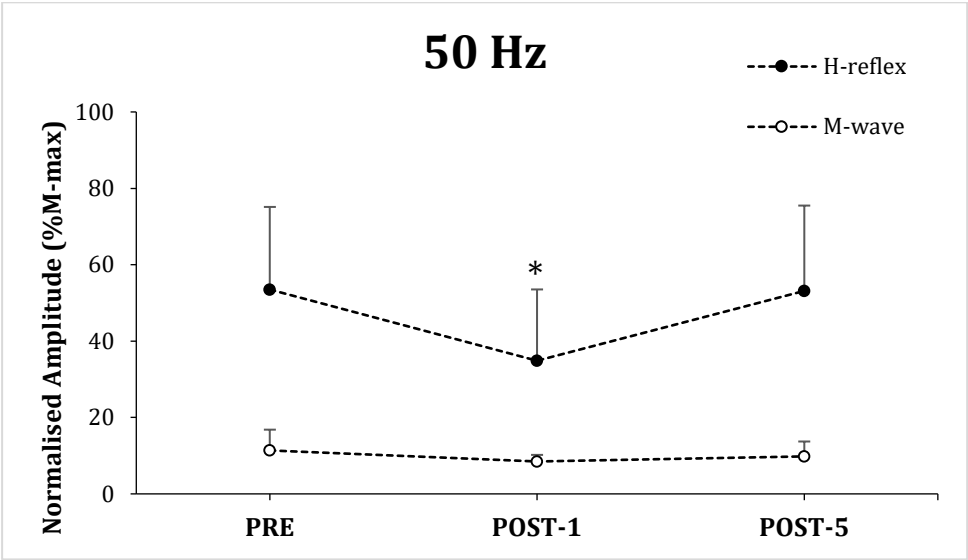

35 Hz
H-reflex
M-wave
Normalised Amplitude (%M-max)
100
80
60
40
20
0
*
PRE
POST-1
POST-5

FIGURE 2D


50 Hz
H-reflex
M-wave
Normalised Amplitude (%M-max)
100
80
60
40
20
0
*
PRE
POST-1
POST-5