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DOES EMG ACTIVATION DIFFER AMONG FATIGUE-RESISTANT LEG MUSCLES DURING DYNAMIC WHOLE-BODY VIBRATION?

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ABSTRACT

The purpose of this study was to determine the effects of dynamic whole-body vibration (WBV) stimuli on the electromyographic (EMG) responses of different fatigue-resistant leg muscles. The participants (N=32) were divided into two groups according to the Fatigue Index value [Group I: Less Fatigue Resistant (LFR), n=17; Group II: More Fatigue Resistant (MFR), n=15]. The repeated EMG activities of four leg muscles [rectus femoris, biceps femoris, vastus lateralis and vastus medialis] were analysed during WBV stimulation. The data were analysed using PASW/SPSS Statistics 18.0 and the significance level was set at $p<0.05$. The results revealed that the vibration frequency, amplitude and group (MFR and LFR) had a significant effect ($p<0.001$) on the EMG response. Dynamic WBV stimuli performed at different frequencies (30Hz, 35Hz and 40Hz) and amplitudes (2mm and 4mm) resulted in significantly increased lower-body muscular activation. However, the LFR group exhibited a significantly higher percentage increase in EMG activation at higher frequencies (max 40Hz) and amplitudes (4mm) ($p\leq0.001$). The results could be useful for the optimal prescription of vibration exercise and can guide the development of training and rehabilitation programmes.

Key words: Fatigue; Fatigue resistance; Isokinetic; Rehabilitation; Muscle activation; Electromyography.

INTRODUCTION

Previous studies of whole-body vibration (WBV) exercise indicate that this form of exercise increases muscle strength (Bosco *et al.*,1999; Torvinen *et al.*, 2002; Delecluse *et al.*, 2003; De Ruiter *et al.*, 2003; Roelants *et al.*, 2004; Cardinale & Wakeling, 2005; Luo *et al.*, 2005; Osawa *et al.*, 2013; Yoosefinejad *et al.*, 2014), enhances the development of movement speed (Cheung *et al.*, 2007; Lamont *et al.*, 2009), improves jumping ability (Lamont *et al*., 2009), improves balance and flexibility (Cochrane & Stannard, 2005; Cheung *et al*., 2007), and positively affect metabolic-hormonal responses in males (Bosco *et al.*, 2000; Kerschan-Schindl *et al.*, 2001; Rittweger *et al.*, 2002; Kvorning *et al.*, 2006). Additionally, WBV stimuli have a favourable effect on the elderly and on individuals with clinical complaints (Schuhfried *et al.*, 2005; Bogaerts *et al.*, 2009; Johnson *et al.*, 2010; Ochi *et al.*, 2015; Orr, 2015). WBV can also be used for therapeutic purposes, such as the prevention of bone loss during rehabilitation (Ezenwa *et al.*, 2008), treatment of spinal cord injuries (Ness & Field-Fote, 2009) and the treatment of multiple sclerosis (Jackson *et al.*, 2008).

WBV exercise has become increasingly popular and has sparked considerable interest, particularly among elite athletes, who perform WBV exercise extensively to increase muscular performance (Bosco *et al*., 1999; Cochrane & Stannard, 2005; Roelants *et al.*, 2006; Abercromby *et al.*, 2007; Di Giminiani *et al.*, 2009; Di Giminiani *et al.*, 2010; Pollock *et al.*, 2010). Increases in muscle performance produced by WBV are theorised to result from the elicitation of involuntary reflex contractions by WBV (Mester *et al.*, 1999) via the tonic vibration reflex (TVR) (Hagbarth, 1967; Burke *et al.*, 1976). The TVR is a spinal reflex that responds to changes in muscle length caused by frequency and/or amplitude displacements generated by the WBV platform. These reflexive contractions might augment voluntary skeletal muscle activation, which results in increased muscle performance (Cardinale & Lim, 2003; Roelants *et al*., 2006).

Krol *et al*. (2011) investigated the muscular activation responses of the *vastus medialis* (VM) and *vastus lateralis* (VL) muscles to different frequencies (20Hz, 40Hz and 60Hz) and amplitudes (2mm and 4 mm). Muscular activation values increased in these muscles due to increases in both the frequency and the amplitude. In another study by Roelants *et al*. (2006) involving three different squat exercises, the maximal voluntary contraction values of leg muscles showed a muscular activation response ranging from 12.6 to 82.4%. Neuromuscular responses of the VL muscle during WBV were explored in a previous study and different frequencies resulted in different muscular activation responses (Cardinale & Lim, 2003).

Hazell *et al*. (2007) investigated the effects of WBV on the electromyographic (EMG) activities of the upper-body (*biceps brachii* and *triceps brachii*) and lower-body muscles [VL and *biceps femoris* (BF)] during isometric semi-squats, dynamic leg squats and static and dynamic bilateral bicep curls. The results of this study demonstrated that: WBV increased muscle activity by 2.9 to 6.7% in the VL and 0.8 to 1.2% in the BF in a static semi-squat; WBV increased muscle activity in the VL by 3.7 to 8.7% and in the BF by 0.4 to 2.0% during dynamic squatting; WBV had no effect on *biceps brachii* EMG activity, but did increase *triceps brachii* EMG activity by 0.3 to 0.7% in a static biceps curl; WBV increased *biceps brachii* EMG activity by 0.6 to 0.8% and *triceps brachii* EMG activity by 0.2 to 1.0% during dynamic bicep curls; and a higher WBV amplitude (4mm) and higher frequencies (35Hz, 40Hz or 45Hz) resulted in the greatest increases in EMG activities (Hazell *et al*., 2007).

Although most of these studies focused on leg muscles, no studies have investigated combinations of these parameters that might allow WBV activation to produce the highest possible level of neuromuscular activity in the leg muscles. The limitations of previous studies include a lack of knowledge of biomechanical variables that determine the vibration load (vibration frequency, vibration amplitude and joint angle) and exercise parameters (side alternating or synchronous vibration platform devices, acute vs. chronic effects, exercise position). These parameters and variables have not been investigated previously. Despite the above-mentioned benefits of vibration stimuli, no effective physical exercise protocol has yet been established for WBV exercises. Additionally, sport that require long or short exercise duration intrinsically require high levels of sensitivity to fatigue and/or recruitment of fatigue-resistant leg muscle groups. No previous studies have investigated the effects of WBV on more fatigue-resistant (MFR) and less fatigue-resistant (LFR) leg muscles. A common conclusion of the previous studies on this topic has been the need to develop an effective personalised vibration training procedure and physical exercise protocol.

PURPOSE OF RESEARCH AND HYPOTHESES

The present study investigated the effects of WBV stimuli at low (2mm), high (4mm) amplitudes and various frequencies (30Hz, 35Hz and 40Hz) on the LFR and MFR groups during dynamic leg squats. It was hypothesised that WBV stimuli applied during dynamic leg squats would cause:

(1) EMG activities of LFR and MFR muscle groups to significantly increase at all frequencies compared with those caused by non-vibrating stimuli;
(2) EMG activity of the LFR leg muscle group to be significantly higher at all frequencies and amplitudes than that of the MFR leg muscle group;
(3) EMG activation of LFR leg muscles to increase by increasing the WBV frequency;
(4) EMG activation of MFR leg muscles to decrease by increasing the WBV frequency; and
(5) EMG activation of MFR and LFR leg muscles to increase by increasing the WBV amplitude.

METHODOLOGY

The University of Osman Gazi Human Research Ethics Board approved this study, and subjects provided their informed written consent prior to participation.

Sample

Physically fit students (N=35) from the Faculty of Sport Science who had no contra-indication associated with WBV, per the manufacturer's recommendations (epilepsy, diabetes, gallstones, kidney stones, acute inflammation, joint problems, cardiovascular diseases, joint inflammation, thrombosis, or back problems such as hernias and tumours), were included in the study. Three of the subjects were excluded because they could not reach 50 repetitions in an isokinetic fatigue protocol. There were 32 subjects who completed the study. The subjects were subjected to an isokinetic fatigue protocol and were divided into 2 groups according to their fatigue index (FI) results (49.99% or less [MFR, n=17]; 50% or more [LFR, n=15]) (Table 1).

TABLE 1. **DESCRIPTIVE DATA FOR MFR AND LFR GROUPS**

Group	N	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
MFR	17	20.13±1.92	178±4.97	72.44±6.76	22.92±1.57
LFR	15	22.64±2.02	179±2.02	77.85±8.64	24.03±1.44

MFR= More Fatigue-Resistant LFR= Less Fatigue-Resistant BMI= Body Mass Index

Experimental design

This investigation used a repeated measures design to determine the effects of dynamic WBV on the EMG responses of different fatigue-resistant leg muscles. The neuromuscular activation [EMG Root Mean Square (RMS)] levels of the knee flexor and extensor muscles served as the dependent variables. The independent variables were vibration frequency

(30Hz, 35Hz and 40Hz), vibration amplitude (2mm and 4mm) and vibration group (MFR and LFR). The study comprised 2 interventions: (1) a fatigue protocol session; and (2) the acquisition of Maximum Voluntary Isometric Contractions (MVICs) and a dynamic WBV session. The order of the test conditions was randomised to control for confounding effects, such as familiarisation or fatigue. Each subject visited the Performance and Biomechanics Laboratory at Anadolu University on 2 separate occasions, with at least 2 days separating successive testing sessions.

Data collection

Isokinetic data acquisition and analyses

In the present investigation, a Cybex isokinetic dynamometer (Humac Norm Testing & Rehabilitation System, Stoughton, MA, USA) was used in the fatigue protocol of knee flexor and knee extensor for the dominant legs of the participating subjects. The experimental session started with a standardised warm-up consisting of 5 minutes of cycling on an ergometer without resistance. Each subject was asked to sit on the chair of the dynamometer with a 90° hip angle and was secured to the chair by a belt to prevent movement of the body. The femoral region of the leg at which the measurements were taken was also fastened to the chair with tape. The region of the knee joint coinciding with the rotational axis was adjusted to the same alignment with the input shaft and the starting point was determined while the knee was anatomically at 0°. The range of motion of the knee joint was set at 90°.

The lower leg was fastened to the lever of the dynamometer. The test position was set as recommended by the manufacturer. Before the test, each subject was advised to achieve the full range of motion during each contraction and to push the isokinetic equipment up to its fullest extension and then to re-pull. An isokinetic familiarisation session was conducted at least 48 hours prior to testing. Before the isokinetic tests, the subjects were asked to perform 3 submaximal concentric contractions at a concentric/concentric angular speed of 180° s^{-1} followed by 2 minutes of rest as a warm-up. During the tests, the subjects were asked to perform 50 reciprocal maximal concentric contractions using the dominant knee at an angular speed of 180° s^{-1}. This angular speed was chosen because it is associated with functional activities that require endurance (Seo *et al.*, 2015). Verbal encouragement and visual feedback of the torque value presented on the monitor were used to motivate for maximum effort.

Isokinetic muscle fatigue was defined as the total muscle work developed in all contractions for each muscle group (knee flexors and extensors). To analyse the isokinetic data, knee flexor and extensor muscle fatigue was determined using 2 calculation methods, namely the FI and the slope. The following formula was used to determine the FI (Thorstensson & Karlsson, 1976):

Percentage decrease = 100-[(work last 3 repetitions/work first 3 repetitions) x100]

The initial peak torque was defined as the mean of the first three peak torque values and the final peak torque as the mean of the last 3 peak torque values. The mean peak torque is the mean value across all 50 repetitions. The calculations and index used by Pincivero *et al*. (1997) was described previously. The slope was determined by means of linear regression by plotting the windowed work values for each repetition across the 50 contractions for each subject. The slope from the calculated regression equation (beta values) for each subject was then calculated to quantify the rate of decrease in quadriceps and hamstring work during the exercise session.

EMG data acquisition and analyses

Surface EMG signals were recorded from the *rectus femoris* (RF), VL, VM and BF muscles of the dominant leg. BF EMG activity was used to represent hamstring muscle function. The recordings were made using a 16-channel wireless EMG system (Delsys Trigno EMG system, Boston, MA, USA). The gain, frequency band, maximum intra-electrode impedance and common noise removal ratio of the EMG amplifier were 1000, 20 to 500Hz, 6kOhm and 95dB, respectively. The EMG signal sampling and bit rates of the analogue-digital converter were 2000Hz and 16bits, respectively.

The measurement sites were prepared by shaving and lightly abrading the area and subsequently cleaning the skin with alcohol. The surface electrodes were placed longitudinally in relation to the underlying muscle fibre arrangement. In accordance with the Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM) recommendations, the centre-to-centre distance between the 2 electrodes was 1cm. In the case of the RF, the sensor was placed at 50% on the line from the *anterior spina iliaca superior* to the superior part of the patella. For the VL, the sensor was placed ⅔ of the way on a line from *the anterior spina iliaca superior* to the lateral side of the patella. For the VM, the sensor was placed 80% of the way on a line between the *anterior spina iliaca superior* and the joint space in front of the anterior border of the medial ligament. In the case of the BF, the sensor was placed midway on a line between the ischial tuberosity and the lateral epicondyle of the tibia (SENIAM).

After the sensor placement session, the MVICs of the muscles were recorded. The MVICs for each muscle were assessed for use as benchmark data. All EMG signals were normalised to the maximum EMG signals recorded during MVICs and presented as the percentage MVIC. MVIC tests were performed with the subject attached to a Cybex isokinetic dynamometer. The MVIC tests of the RF, VL and VM were based on knee extension in a seated position, with the knee at 65° (0° full extension), whereas the MVIC tests of the hamstrings (long head of the BF) were assessed in a prone position with the knee at 30° (Kellis & Baltzopoulos, 1998). The subjects performed 1 trial to understand the task and then performed 2 repetitions, each 5 seconds in duration. During the test, the subjects received standardised verbal motivation to produce maximum effort. A 2-minute rest was allowed between contractions.

Prior to the experimental protocol, a familiarisation session was performed with all subjects to familiarise them with the sensations associated with WBV. The vibration measurements were made using a Compex WINPLATE Galileo 2000 (Novotec Medical GmBH, Germany). The subjects were asked to stand on a WBV platform and were then directed to assume a dynamic squat position with a knee flexion angle between 90° and 150° and with their arms flexed at approximately 90°. Joint angle measurements were collected with a goniometer to ensure that the subjects maintained the required position. A dynamic squat was performed starting from an upright posture with approximately 150° knee flexion. The subjects squatted slowly until approximately 90° knee flexion was achieved. To control the angular velocity of the flexion and extension movements, a test operator used a metronome at 60bpm concurrently with verbal commands such that the flexion and extension phases of movement each lasted 2 seconds, with a 1-second pause between phases. The subjects were provided verbal feedback about the joint position throughout the protocol and the movement position was self-corrected. The foot position on the platform was marked during the initial trial of each experimental session and the subjects were asked to maintain their foot position during all trials. The subjects were instructed to direct their head and eyes forward and to distribute their weight equally on both feet.

At the beginning of each measurement, baseline EMG activity (non-vibration) was measured while the subjects were performing a dynamic squat. This procedure allowed an assessment of the contribution of vibration to EMG activity. Baseline EMG measurements were then obtained for 30 seconds and subjects were randomly exposed to 6 different test conditions (frequency [3] x amplitude [2]) on a vibration platform. Each test condition lasted for 30 seconds, with 5 minutes rest between each condition and 10 minutes rest before the 4mm amplitude condition to prevent fatigue.

With the aid of the MATLAB (MathWorks, R2012a), the EMG data was processed. The EMG signals were band-pass filtered (20-450Hz) and smoothed using the RMS values and a 500ms moving-window function (Harput *et al.*, 2013; Harput *et al.*, 2014). Motion artefact components of recorded EMG signals were filtered out using an infinite impulse-response notch filter (3dB band=1.5Hz) centred on the applied vibration stimulus frequency and its harmonics (Fratini *et al.*, 2009). Notch filters were applied for all recordings except the EMG of the resting periods.

Data analysis

The Standard Error of Measurement (SEM) and the corresponding 95% confidence intervals were calculated and subsequently expressed in the units of each variable. Independent-sample t-tests were performed between the calculated FIs for the 2 groups for each isokinetic variable and movement. Values are presented as the mean±standard error (SE). Before statistical analyses, all of the EMG measures were found to be distributed normally based on the Shapiro-Wilk test. The dependent variables in all of the statistical tests were EMG values measured from the RF, VM, VL and BF muscles. The independent variables included the randomisation group (MFR and LFR), vibration frequency (30Hz, 35Hz and 40Hz) and vibration amplitude (2mm and 4mm). Repeated measures analysis of variance (ANOVA) (group [2] x frequency [3] x amplitude [2]) was applied to detect interaction effects between the independent variables. A Bonferroni post hoc test was applied to all pair wise comparisons when a significant result was found. The vibration data were analysed using PASW/SPSS Statistics 18.0 (SPSS Inc., Chicago, IL) and the significance level was set at $p<0.05$.

RESULTS

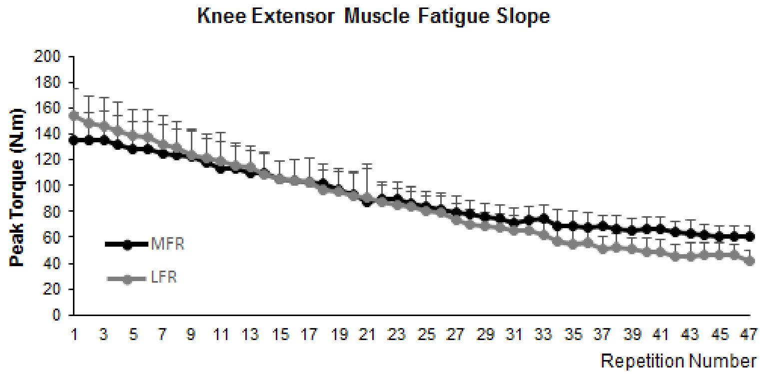
Descriptive data regarding the single highest repetition values (mean and SD) for absolute peak torque (Nm), absolute mean torque (Nm) and relative mean torque values ($Nm.kg^{-1}$) of the leg extensor and flexor muscles for both the MFR and the LFR groups are presented in Table 2.

TABLE 2. **SINGLE HIGHEST REPETITION VALUES (MEAN±SD) FOR ABSOLUTE PEAK TORQUE (Nm), ABSOLUTE MEAN TORQUE (Nm) AND RELATIVE MEAN TORQUE VALUES (Nm.kg^{-1})**

Variables	**Group**	**N**	**Leg Extensor** Mean±SD	p-Value	**Leg Flexor** Mean±SD	p-Value
Absolute peak torque (Nm)	MFR	17	139.16±4.62	0.019*	95.30±5.01	0.014*
	LFR	15	158.46±7.48		106.10±5.45	
Absolute mean torque (Nm)	MFR	17	86.53±8.41	0.001**	64.06±6.70	0.001**
	LFR	15	145.73±7.50		96.06±5.73	
Relative mean torque (Nm.kg^{-1})	MFR	17	119.60±12.03	0.001**	87.00±10.94	0.007*
	LFR	15	192.40±6.49		128.00±7.28	

*p<0.05 **p<0.001

As shown in Table 2, the LFR group had significantly higher absolute peak torque, absolute mean torque and relative mean torque values of the leg extensor muscles when compared with the MFR group. The LFR group also had significantly higher absolute peak torque, absolute mean torque and relative mean torque values of the leg flexor muscles when compared with the MFR group.



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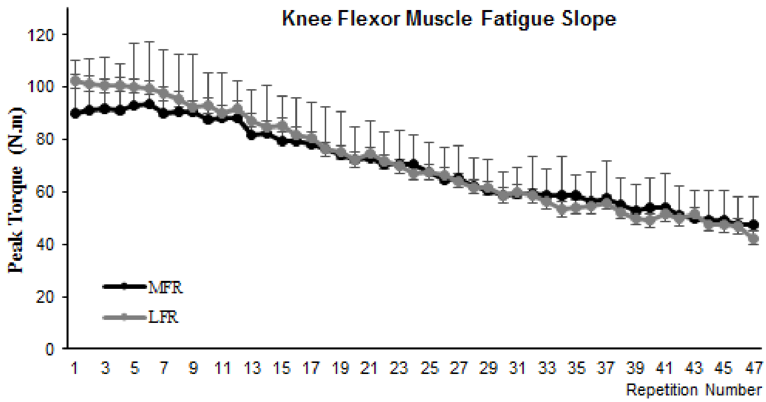

FIGURE 1. **LINEAR SLOPE FOR MEAN KNEE EXTENSOR AND FLEXOR PEAK TORQUE DURING 50 MAXIMAL-EFFORT CONCENTRIC REPETITIONS**

Figure 1 shows the decrease in work output, measured as the linear slope for the knee extensor and flexor muscles of the MFR and LFR groups. The findings of the present study demonstrate that the LFR group experienced a significantly higher rate of muscle fatigue of the knee extensor and knee flexor when compared with the MFR group ($p\leq0.001$). Table 3 and Table 4 indicate the knee extensor and flexor FI and slope rates, respectively.

TABLE 3. **DESCRIPTIVE DATA OF KNEE EXTENSOR AND FLEXOR FATIGUE INDEX (FI%) FOR MFR AND LFR GROUPS**

Group	**n**	**Knee extensor** Mean±SD	**95% CI**	**p-Value**	**Knee flexor** Mean±SD	**95% CI**	**p-Value**
MFR	17	46.08±2.29	42.52-49.64	p≤0.001	32.43±3.51	25.21-38.73	p≤0.001
LFR	15	69.54±1.33	67.15-72.14	p≤0.001	56.59±3.41	44.28-57.77	p≤0.001

TABLE 4. **DESCRIPTIVE INFORMATION REGARDING KNEE EXTENSOR AND FLEXOR SLOPE (Nm.rep^{-1}) FOR MFR AND LFR GROUPS**

Group	**n**	**Knee Extensor** Mean±SD	**95% CI**	**R^2**	**p-Value**	**Knee Flexor** Mean±SD	**95% CI**	**R^2**	**p-Value**
MFR	17	-0.54±1.53	-0.51-(-0.57)	0.96	p≤0.001	-0.87±1.40	-0.83-(-0.91)	0.98	p≤0.001
LFR	15	-0.40±0.9	-0.38-(-0.42)	0.97	p≤0.001	-0.73±1.37	-0.69-(-0.76)	0.97	p≤0.001

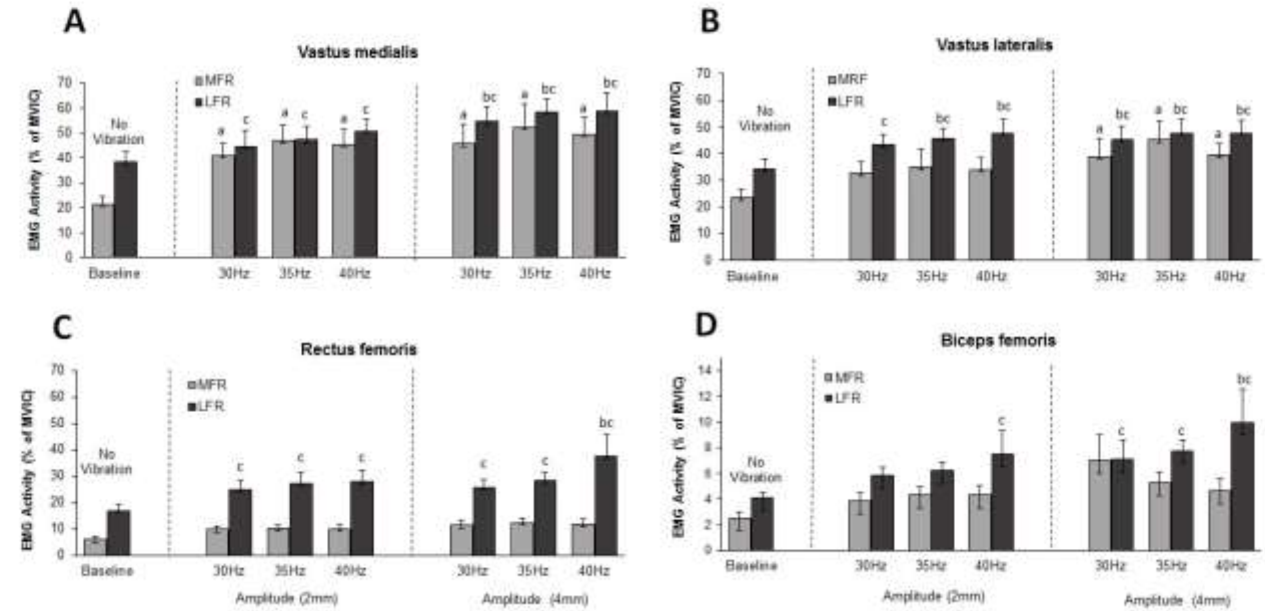

FIGURE 2 (A-D). **INCREASES IN MUSCLE ACTIVITY WITH WBV COMPARED WITH NON-WBV DURING A DYNAMIC SQUAT**

Black and open bars show the EMG activity of the LFR and MFR groups, respectively, at different frequencies and amplitudes.
Values are the mean±SE. Significantly greater than baseline: $p<0.05$.
Different letters (a, b, c) indicate significant differences in activity levels between frequencies during either high- or low-amplitude vibration.

MFR Group Baseline-MFR Group:	30, 35, 40Hz (2mm) / 30, 35, 40Hz (4mm)	**a** ($p<0.05$).
LFR Group Baseline-LFR Group:	30, 35, 40Hz (2mm) / 30, 35, 40Hz (4mm)	**b** ($p<0.05$).
MFR Group Baseline-LFR Group:	30, 35, 40Hz (2mm) / 30, 35, 40Hz (4mm)	**c** ($p<0.05$).

Figure 2 shows the increases in VM, VL, RF and BF muscle activity with WBV compared with non-WBV and the differences between the LFR and MFR groups with respect to EMG activities for all frequencies and amplitudes during a dynamic squat.

VM muscle

Figure 2-A represents the VM muscle activity. The VM EMG RMS activity was consistently higher in the WBV condition when compared with the non-vibration condition. Statistical analyses confirmed significant main effects of 'group' ($F_{(1,330)}$=77.41; p<0.001), 'frequency' ($F_{(3,330)}$=27.52; p<0.001) and 'amplitude' ($F_{(2,330)}$=65; p<0.001) on the EMG response. In addition, significant interaction effects were found for 'group x amplitude' ($F_{(2,330)}$=5.54; p<0.001) and 'frequency x amplitude' ($F_{(6,330)}$=7.59; p<0.001). However, 'group x frequency' and 'group x amplitude x frequency' interactions were not significant (p>0.05).

VL muscle

As shown in Figure 2-B, the EMG RMS activity of the VL muscle was consistently higher in the WBV condition than in the non-vibration condition. Statistical analyses confirmed significant effects of 'group' ($F_{(1,330)}$=118.07; p<0.001), 'frequency' ($F_{(3,330)}$=24.87; p<0.001) and 'amplitude' ($F_{(2,330)}$=59.28; p<0.001) on the EMG response. In addition, a significant interaction effect was determined for 'frequency x amplitude' ($F_{(6,330)}$=6.95; p<0.001), but not for 'group x frequency', 'group x amplitude' nor 'group x amplitude x frequency' interactions (p>0.05).

RF muscle

Figure 2-C represents the activity of the RF muscle. The RF EMG RMS activity was consistently higher in the WBV condition than in the non-vibration condition. Statistical analyses confirmed significant effects of 'group' ($F_{(1,330)}$=311.66; p<0.001), 'frequency' ($F_{(3,330)}$=5.06; p<0.001) and 'amplitude' ($F_{(2,330)}$=10.48; p<0.001) on the EMG response. There were no significant effects for 'group x amplitude', 'group x frequency', 'frequency x amplitude' and 'group x amplitude x frequency' interactions (p>0.05).

BF muscle

Figure 2-D represents the activity of the BF muscle. BF EMG RMS activity was consistently higher, although the difference was not always significant in the WBV condition when compared with the non-vibration condition. Statistical analyses confirmed significant effects for 'group' ($F_{(1,330)}$=31.55; p<0.001), 'frequency' ($F_{(3,330)}$=7.38; p<0.001) and 'amplitude' ($F_{(2,330)}$=19.54; p<0.001) on the EMG response. In addition, an interaction effect of 'frequency x amplitude' ($F_{(6,330)}$=6.95; p<0.001) on the EMG response was found, but not for 'group x amplitude', 'group x frequency' and 'group x amplitude x frequency' interactions (p> 0.05).

The results showed that the all recorded leg muscles were affected differently by the different amplitudes, frequencies and groups:

- Significant *group* main effects were found, revealing that the EMG activity of the LFR group was significantly higher than that of MFR leg muscle group (p<0.05).

- Significant *amplitude* main effects were found revealing that the 4mm amplitude resulted in significantly greater EMG activity than the 2mm amplitude did in all tested muscles ($p<0.001$).
- Significant *frequency* main effects were found, revealing that compared with non-WBV, WBV resulted in significantly greater EMG activity in all tested muscles ($p<0.001$).
- Significant *interaction* effects were observed between the amplitude and vibration frequency (amplitude x frequency) with respect to the EMG activities of the VM, VL and BF ($p<0.001$). In the LFR group, the highest frequency (40Hz) and amplitude (4mm) resulted in significant increases in EMG activities in the VM, VL, RF ($p\leq0.001$) and BF ($p<0.05$). The highest level of muscular activation in the MFR group was observed at the frequency of 35Hz and amplitude of 4mm and thereafter tended to decrease in the VM, VL, RF and BF. However, these decreases were only significant for the VM and VL ($p<0.001$).

DISCUSSION

Most of the studies previously conducted on this topic have focused on the acute and chronic effects of WBV on neuromuscular performance. Although interest in WBV is steadily increasing, little is known about the neuromuscular mechanism through which WBV acts, about how the human body responds to WBV or about the type of WBV stimulus (frequency x amplitude) that would be optimal for the development of the most beneficial individual treatment protocols. In some studies, muscle strength increased (Bosco & Komi, 1979; Torvinen *et al*., 2002; Roelants *et al*., 2004; Delecluse *et al.*, 2005), whereas in other studies muscle strength did not change (Delecluse *et al*., 2003; De Ruiter *et al*., 2003; Cochrane & Stannard, 2005). According to the findings of these studies, individual differences are found across subjects. Thus, the response to the applied combination of frequency and amplitude could be different, causing controversial issues related to applications. In this study, to decrease the individual differences in the subjects, all subjects were grouped according to the level of fatigue resistance of leg muscle. Aside from these studies, no other studies investigating the effects of dynamic WBV on the EMG responses of different fatigue-resistant leg muscles could be found in the literature. Considering the information above, the aim of the present study was to investigate the effects of WBV stimuli at low (2mm) and high (4mm) amplitudes and at various frequencies (30Hz, 35Hz and 40Hz) on the EMG responses of different fatigue-resistant leg muscles during dynamic leg squats.

The first hypothesis of this study was confirmed. The muscle activities of the LFR and MFR groups significantly increased at all WBV frequencies compared with those at the non-vibration baseline measurement. Most studies investigating WBV exercise protocols implemented frequencies between 15Hz and 60Hz (Cardinale & Wakeling, 2005). Surface EMG analyses in different studies have demonstrated significantly increased muscular activity and several studies have reported that the EMG increase is frequency-dependent (Matthews, 1966; Issurin & Tenenbaum, 1999; Bosco *et al*., 2000; Cardinale & Lim, 2003; Luo *et al*., 2005; Roelants *et al*., 2006; Hazell *et al*., 2007; Fratini *et al*., 2009; Pollock *et al*., 2010; Di Giminiani *et al.*, 2013). Matthews (1966) suggested that the level of muscle response to mechanical vibration depends on the applied frequency and that higher frequencies would lead to greater muscle activity.

The findings of the study by Cardinale and Jin (2003) revealed higher EMG activity with WBV when compared to the non-vibrating condition, supporting the results of the present study. Hazell *et al*. (2007) investigated the effects of WBV during dynamic and static contractions of the upper and lower body. These authors showed that WBV stimuli at different frequencies and low or high amplitudes caused significant increased EMG RMS muscle activity. In particular, frequencies from 35 to 45Hz and frequencies with higher amplitude (4mm) resulted in greater muscular activation. In another study, Pollock *et al*. (2010) examined the effects of frequency and amplitude on the activity and acceleration value of six leg muscles during WBV. They observed that the muscular activation values were greater, although not always significant, at high amplitudes and at all frequencies.

Perchthaler *et al*. (2013) reported changes in the neuromuscular activity of thigh muscles during WBV by incorporating various biomechanical variables. These findings showed that different frequency and amplitude combinations resulted in different ratios of EMG percentage MVICs. Muscular activation increased linearly with increasing frequency, except in the *gluteus maximus* and BF muscles (Perchthaler *et al*., 2013). Krol *et al*. (2011) suggested that the level of muscle response to mechanical vibration depended on the applied frequency and that higher frequencies would lead to greater muscle activity. These improvements in muscle performance following acute WBV exposure are considered to be associated with numerous different mechanisms.

The most often cited mechanism proposed to explain these effects of WBV exposure is the 'tonic vibration reflex' (TVR). The higher frequency and amplitude of the EMG signal obtained from the abdomen of the examined muscles indicate a larger number of simultaneously stimulated motor units, as well as a better synchronisation of their stimulation (Hazell *et al*., 2007; Cardinale & Erskine, 2008). The TVR produces involuntary rapid changes in muscle length and increases muscle activation (Hagbarth & Eklund, 1966) via vibration perturbation. Furthermore, the recruitment thresholds of motor units decrease (Romaiguere *et al*., 1993) and activating a larger portion of the motor unit pool (Issurin & Tenenbaum, 1999), augments EMG. These mechanisms indicate that exposure to WBV can stimulate the neuromuscular system. In fact, the results of these studies show that WBV elicits higher EMG activity than the non-vibrating condition. However, different vibration frequencies elicit different EMG responses in the stimulated muscles.

The second hypothesis of this study was confirmed because in the comparison of different fatigue-resistant leg muscles, the LFR group demonstrated higher muscle EMG activation than the MFR group at all frequencies. Under exposure to WBV, the highest EMG activity in the LRF group was recorded when the frequency was 40Hz. Thus, the highest frequency elicited the greatest reflex response in the LFR muscle during dynamic WBV. Therefore, a stimulus with a higher frequency may potentially lead to improvements in the performance of LFR group leg muscles. However, the highest level of muscular activation in the MFR groups was observed at a frequency of 35Hz. The muscle activation of the MFR group decreased as the WBV frequency increased. This finding may be associated with the different characteristics of absolute torque production of the LFR and MFR groups. During 50 maximal-effort concentric repetitions, the LFR group displayed significantly higher absolute peak torque (Nm), absolute mean torque (Nm) and relative mean torque values ($Nm.kg^{-1}$) for the extensor and flexor leg muscles than did the MFR group. This finding can be explained by the individual differences in the number of myofibrils in each muscle fibre, muscle fibre recruitment and frequency; the number of sarcomeres in series; and the arrangement of muscle fibres relative to the axis of force generation, that is the muscle architecture (Enoka, 2008).

Vibration amplitudes are important variables that affect muscle activity during vibration stimuli (Martin & Park, 1997). The literature reports amplitudes ranging from 2mm to 10mm (Cardinale & Wakeling, 2005). However, the selective effects of different vibration-amplitude parameters are not clearly understood. A wide variety of frequencies, amplitudes and durations of exposure has been used in different study protocols. Krol *et al*. (2011) suggested that EMG activity significantly increased with both amplitude and frequency, with EMG activity reaching its highest level when the frequency and amplitude reached 60Hz and 4mm, respectively. The same vibration frequency at different amplitudes (2mm and 4mm) resulted in significantly higher EMG activity at 4mm. The findings of the present study showed that a significant 'amplitude' effect was found on the EMG response of LFR and MFR leg muscles. In comparing different amplitudes, the higher amplitude (4mm) demonstrated greater muscle activation of LFR and MFR leg muscles.

From a practical point of view, lower frequencies and higher amplitudes (35Hz and 4mm, respectively) of vibration may enable individuals with MFR muscles to achieve maximal muscular activation. However, the EMG data from this study demonstrate that higher WBV frequencies and amplitudes (40Hz and 4mm) elicit the greatest increases in muscle activity with LFR muscles and emphasise that selection of the correct frequency and amplitude is likely critical for optimal performance improvement.

CONCLUSION

In conclusion, the objective of this study was to evaluate the effects of dynamic WBV stimuli on the EMG responses of different fatigue-resistant leg muscles. The results show that dynamic WBV has a positive effect on muscle EMG activities, which are dependent on the vibration frequency and amplitude and the group (MFR and LFR). Based on these results, the present study can be used as a reference to pave the way for the development of an effective individual WBV exercise program, including primary assessment of the individual's or athlete's muscle fatigue index and determining the training intensity to reach the optimal vibration stimulus for the LFR or MFR leg muscles.

Finally, identifying an optimal stimulus resulting in the greatest increase in muscle activity should aid in the development of optimal vibration exercise prescriptions and can serve as a guide for the development of training or rehabilitation programmes. The optimal frequency, optimal amplitude and muscle activation level that would benefit most from vibration stimulation must all be identified to induce specific adaptations. Moreover, different fatigue-resistant leg muscle groups react differently to the applied frequency. This implies grouping the vibration frequency according to the ratio of fatigue resistance that can elicit the greatest reflex response during WBV. WBV performed according to the ratio of fatigue resistance may boost performance in a shorter time compared with WBV performed using a fixed frequency.

RECOMMENDATIONS

This study has some methodological limitations. Three vibration frequencies of 30Hz, 35Hz and 40Hz and two amplitudes of 2mm and 4mm were applied. Muscular activation values of the LFR group were highest at a frequency of 40Hz. Further studies should be conducted to determine the muscular activation pattern at higher frequencies (45Hz and higher) for individuals with LFR leg muscles and at higher amplitudes for individuals with LFR and MFR leg muscles. Another limitation was the squat position. This application has been performed only in a dynamic squat position. Future studies that use different knee flexion angles and a static squat position are needed. In this study, the LFR and MFR groups were determined according to isokinetic torque responses. Further studies should determine the ratio of distribution of fatigue-resistant fast-twitch (type II) and fatigue-sensitive slow-twitch (type I) fibres with biopsy or phosphorus magnetic resonance spectroscopy methods. The LFR and MFR groups can be determined according to the composition of muscle fibre type because of individual responses and the effectiveness of individualised treatments on neuromuscular performance.

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