

Effects of low frequency whole body vibration on motor evoked potentials in healthy men

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Abstract

The aim of this study was to determine whether low frequency whole body vibration (WBV) modulates the excitability of the corticospinal and intracortical pathways related to tibialis anterior (TA) muscle activity thus contributing to the observed changes in neuromuscular function during and after WBV exercise. Motor evoked potentials (MEPs) elicited in response to transcranial magnetic stimulation (TMS) of the leg area of the motor cortex were recorded in TA and soleus (SOL) muscles of 7 healthy male subjects whilst performing 330s continuous static squat exercise. Each subject completed 2 conditions: control (no WBV) and WBV (30Hz, 1.5mm vibration applied from 111s to 220s). Five single suprathreshold and five paired TMS were delivered during each squat period lasting 110s (pre-, during and post-WBV). Two interstimulus intervals (ISI) between the conditioning and the testing stimuli were employed in order to study WBV effects on short-latency intracortical inhibition (SICI, ISI=3ms) and intracortical facilitation (ICF, ISI=13ms). During vibration relative to squat exercise alone, single pulse TMS provoked significantly higher TA MEP amplitude ($56\pm 14\%$, $p=0.003$) and total area ($71\pm 19\%$, $p=0.04$) and paired TMS with ISI=13ms provoked smaller MEP amplitude ($-21\pm 4\%$, $p=0.01$) but not in SOL. Paired pulse TMS with ISI=3ms elicited significantly lower MEP amplitude (TA: $-19\pm 4\%$, $p=0.009$; SOL: $-13\pm 4\%$, $p=0.03$) and total area (SOL: $-17\pm 6\%$, $p=0.02$) during vibration relative to squat exercise alone in both muscles. TA MEP facilitation in response to single pulse TMS suggests that WBV increased corticospinal pathway excitability. Increased TA and SOL SICI and decreased TA ICF in response to paired pulse TMS during WBV indicate vibration-induced alteration of the intracortical processes as well.

Introduction

Brief (<20min daily) low frequency (10 to 50Hz) vibration stimulation transmitted to the whole body or part of it during sub-maximal exercise elicits acute neural adaptations (Mileva *et al.*, 2006; Roelants *et al.*, 2006) and chronic strength gains (Delecluse *et al.*, 2003) similar to those produced by conventional resistance strength training. These low vibration frequencies fall within the range of the natural resonant frequencies for different body segments and tissues and their transmission through the body segments differs from that of higher frequency (>60 Hz) vibrations (Wakeling *et al.*, 2002; Mester *et al.*, 2006; Gupta, 2007). Acute stimulation with low frequency vibration induces transient increases in the electrical activity of the vibrated muscle during submaximal dynamic and isometric (static) contractions (30-50Hz Cardinale & Lim, 2003; 35Hz Roelants *et al.*, 2006; 25-45Hz, Hazell *et al.*, 2007) as well as in sub-maximal (30Hz, Bosco *et al.* 1999) and maximal (10 Hz, Mileva *et al.*, 2006) movement power. Simultaneous vibration and stretching were shown to induce acute increases in flexibility whilst maintaining explosive strength (30Hz Kinser *et al.*, 2008). A single session of whole body vibration (WBV) during static squat exercise has also been shown to produce clinical benefits including improved postural control, mobility and balance in multiple sclerosis patients with moderate disability (1-4.4Hz, Schuhfried *et al.* 2005) and in patients with Parkinson's disease (6Hz, Haas *et al.* 2006).

Chronic whole limb or whole body vibration training is able to induce: 1) a similar degree of chronic isometric and dynamic strength enhancement as moderate intensity resistance training and significantly higher increases in explosive strength (35-40Hz, Delecluse *et al.* 2003); 2) improvement of gait and body balance in elderly patients (10 and 26 Hz, Bruyere *et al.* 2005); 3) attenuation of calf muscle atrophy after prolonged immobilisation (19-25Hz, Blottner *et al.* 2006). However, the magnitude of vibration effects varies across studies and in some cases acute vibration stimulation has resulted in decreased (Rittweger *et al.* 2000) or

unchanged (Torvinen *et al.* 2002) muscle functional performance immediately post-exercise. Chronic WBV consistently improves muscle performance when compared to a passive control group, however 4 out of 5 studies found no effect of WBV when responses were compared to a control group performing identical exercise without WBV (for detailed review see Nordlund & Thorstensson, 2007). Most likely this variation is due to the wide range in vibration intensities (frequency and amplitude) and exercise modes employed. The growing use of WBV for rehabilitation from muscle and neurological injury and its use by athletes to improve muscle strength necessitate an improved understanding of how this mechanical stimulus interacts with the human neuromuscular system since neither the functional effects of WBV nor the mechanisms of such effects have yet been fully characterised.

There is a considerable body of published work utilising high frequency muscle and tendon vibration (HFV, >60 Hz) as a tool to study sensorimotor integration in health and disease. High frequency direct muscle/tendon vibration seems to primarily activate the Ia afferents of the muscle spindles and to a lesser degree the Golgi afferents (Ib) and secondary spindle afferents (Roll *et al.* 1989). The spinal circuitry is the first stage within the motor feedback loop for generating fast efferent reactions in response to proprioceptive input although central projections from supraspinal motor centers also control such reactions (Chez & Krakauer, 2000). Cortical areas also receive and process proprioceptive information and accordingly generate evoked cortical potentials in response to direct high frequency vibration (Munte *et al.* 1996). Muscle afferent input to the cerebral cortex appears to play a major role in motor control (Wiesendanger & Miles, 1982) and facilitation from muscle afferents may contribute up to 30% of central motor drive (Macefield *et al.* 1993). It has been demonstrated in humans that altered Ia afferent input can change the excitability of the corticospinal pathway (Carson *et al.* 2004), as well as the activation of cortical motor regions (Lewis *et al.* 2001). The excitability of the intracortical inhibitory systems is also influenced by changes in

afferent input (Ridding *et al.* 2005). Direct muscle/tendon vibration has been shown to entrain the Ia afferent firing rate in a linear fashion at frequencies up to 70–80Hz (Roll *et al.* 1989). Therefore, alterations of peripheral reflexes as well as of segmental and corticospinal processes are candidate mechanisms for the observed functional effects of low frequency WBV.

Transcranial magnetic stimulation (TMS) of the human motor cortex provides a method for studying the excitability of the corticospinal system, as well as intracortical inhibitory and facilitatory processes. Significant augmentation of motor-evoked potentials elicited by TMS has been observed when 80Hz vibration was applied to extensor carpi radialis muscle, which suggests that vibration increases motor cortex excitability (Siggelkow *et al.* 1999; Kossev *et al.* 2001). Targeted high frequency vibration of the muscle or tendon has also been shown to reduce short-interval intracortical inhibition (Rosenkranz & Rothwell, 2006), whilst the opposite occurs within neighbouring and contralateral muscles (Rosenkrantz & Rothwell, 2003). Alteration of cortical excitability induced by muscle tendon vibration demonstrates non-linear frequency dependency with greater MEP potentiation at 75 vs. 20 and 120 Hz (Steyvers *et al.* 2003); and at 80 vs 120 and 160 Hz (Siggelkow *et al.* 1999). Thus, it is of interest to explore the effects of the proprioceptive input induced by low frequency whole body vibration on the corticospinal and intracortical processes. TMS studies have focused on the responses evoked in upper limb muscles (Siggelkow *et al.*, 1999; Kossev *et al.*, 2001, 2003; Rosenkrantz & Rothwell, 2003; 2006). Although the time course of the responses to TMS of the motor cortex area representing lower limb muscles has not yet been studied systematically, MEPs following single and paired TMS show similar characteristics to those described for the hand motor area (Stokic *et al.* 1997). Therefore, the project aim was to investigate the effects of WBV during static

squat exercise on corticospinal excitability and intracortical processes by studying motor evoked potentials (MEPs) in the shank muscles, in response to single and paired pulse transcranial magnetic stimulation. In contrast to direct muscle or tendon vibration, during WBV all movement agonist and antagonist muscles are simultaneously subjected to the stimulus. Therefore, muscle responses evoked by TMS during WBV exercise will be examined in parallel in two antagonist ankle stabiliser muscles - tibialis anterior (TA) and soleus (SOL). The TMS protocol will be optimised to obtain primarily MEPs in the TA muscle because the corticospinal projections to the TA are shown to be the strongest amongst all leg muscles (Brouwer & Ashby, 1992; Perez *et al.*, 2004).

Methods

Subjects

Seven healthy male adults (mean \pm SD, n=7; 36 \pm 11yrs, 181 \pm 9cm, 82 \pm 13kg) with no previous motor disorders or current injuries and taking no medication gave their written informed consent to participate in this study. The protocol of the study was approved by the local university ethics committee and was performed according to the Declaration of Helsinki. Subjects were recruited from the student/staff population at the university. One of the subjects was not involved in any type of regular physical activity, the remaining 6 subjects were recreationally active: moderate intensity gym based training (n=3); high intensity gym based training and cycling (n=2), intensive outdoor cycling (n=2).

Experimental protocol

Each subject (n=7) attended the laboratory on 3 occasions: once for familiarisation procedures and twice for completion of the four main trials, with at least 3 days between

visits. Two main trials were completed during each visit, with the first trial on each occasion a control trial with either short-latency intracortical inhibition (SICI) or intracortical facilitation (ICF). SICI and ICF were investigated using techniques previously developed and described by other researchers (Kujirai *et al.*, 1993; Kossev *et al.*, 2001, 2003; Perez *et al.*, 2004; Ridding *et al.*, 2005). These techniques are briefly described in the sections below. To avoid the confounding effects of experimental fatigue, the trial was repeated (SICI or ICF) after at least 30min of seated rest with vibration applied during the second static squat period (WBV at 30Hz frequency and 1.5mm vibration amplitude). The order of the trials (SICI or ICF) for different subjects in the study was allocated by systematic rotation to counteract any order effect.

During the preliminary visit subjects were familiarized with the protocol and equipment. Subjects were specifically instructed and trained to maintain identical posture and to distribute their body weight evenly over the foot throughout the trials. TA resting motor threshold (MT) was determined as the lowest TMS intensity required to elicit a MEP of minimum 50 μ V peak-to-peak amplitude in at least 3 out of 5 single consecutive stimulations at that intensity from the relaxed muscle (Perez *et al.*, 2004). The subject was seated in a chair with knee joint angle of 110⁰ (approximates neutral seated position) and asked to keep the feet flat and relaxed on the floor. The muscle relaxation was monitored by continuous display of the background EMG activity recorded from the TA and SOL muscles. MT determination was performed in two stages – first, to identify the region of lower limb muscle representation of the motor cortex, and second, to determine the optimal stimulus intensity. MT was also tested and confirmed at the start of each main trial.

Each main trial consisted of 330s continuous static squat exercise at 30⁰ knee flexion (Fig.1). Vibration was applied from 111s to 220s (termed period 2 or during WBV) in the WBV trial only. No vibration was applied in either trial from 0s to 110s (termed period 1 or pre-WBV)

or from 221s to 330s (termed period 3 or post-WBV). During one of the visits, subjects received alternating single pulse (5 repeats) and paired pulse TMS with inter-stimulus interval (ISI) of 3ms (5 repeats, SICl) during each stage of the exercise protocol (Fig.1) in both trials (control and vibration). The same experimental protocol was applied during the other laboratory visit except that a longer inter-stimulus interval was applied for the paired pulse TMS (13ms, ICF). Vibration stimulation (30Hz, 1.5mm peak-to-peak amplitude protocol) was delivered by standing on a vibrating platform (FitVibe Medical, Uniphy Elektromedizin GmbH &Co KG, Germany). The output of the platform during this protocol was measured in pilot trials and found to produce vertical sinusoidal acceleration at 30Hz with vertical displacement of 1.63 ± 0.09 mm. The subjects were wearing only socks to prevent damping of the stimulus in the shoe soles. Subjects placed their feet shoulder width apart on the platform and kept their arms crossed above their chest in order to avoid using them for postural support during the trial. Subjects were reminded to assume their normal posture as established during the familiarization visit, and visual feed-back from the knee electrogoniometer was provided on a monitor.

Data recording

Surface EMG activity and the motor evoked potentials were recorded from TA and SOL muscles of the right leg using active bipolar electrodes (99.9% Ag, 10mm length, 1mm width, 10mm pole spacing, CMRR>80dB, model DE2.1, DeISys Inc, Boston, MA). The electrode for recording TA EMG activity was placed proximally over the muscle belly parallel to the longitudinal axis of the muscle. The electrode for SOL EMG recording was placed centrally over the lateral portion of the muscle and oriented at an angle of 45° (relative to the midline of the posterior aspect of the shank connecting the Achilles tendon insertion and the popliteus

cavity) to approximate the muscle fibre pennation angle. The ground electrode was placed over the patella of the right leg.

Knee joint angular displacement profile (flexion/extension) was recorded continuously via a pre-amplified bi-axial electrogoniometer (Biometrics system, Gwent UK), which was attached with double-sided medical tape to the lateral surface of the right leg. The device was centred over the lateral epicondyle of femur with one endplate attached to the shank and aligned to the lateral malleolus of fibula and the other – to the thigh and aligned to the greater trochanter of femur. The knee flexion angle was set to zero at 180° angle between the femur and the fibula, which approximates neutral standing position. During each trial subjects were provided with continuous visual feedback on their knee angular position in order to keep constant posture.

The EMG signals were amplified (x1000), band-pass filtered between 20-500Hz (Bagnoli-8, DelSys Inc, Boston, MA) and transferred on-line to a computer with a sampling frequency of 2kHz. The signal from the electrogoniometer was pre-amplified in the conditioning unit mounted on a subject's belt and sampled with a frequency of 200Hz. EMG and electrogoniometry data were recorded continuously and digitised synchronously via an analogue-to-digital converter (CED 1401power, Cambridge, UK), using Spike2 data acquisition software (CED, Cambridge, UK) with a resolution of 16 bit.

Transcranial magnetic stimulation

Motor evoked potentials (MEP's) in the shank muscles were elicited by TMS of the contralateral motor cortical leg area. The stimulation was provided by a pair of Magstim 200 stimulators (Magstim Co Ltd,UK) producing pulses of 100µs duration and up to 2T intensity. The stimulators were triggered by a Bistim unit (Magstim Co Ltd,UK) which allows adjustment of the interval between the generated TMS pulses. The TMS pulses were delivered to the

motor cortex through a 110⁰ double cone coil (9cm diameter each, type P/N 9902-00, Magstim Co Ltd, UK). The coil was centred over the scalp in the area of the vertex so that the posterior-to-anterior current flow from the two coils overlapped the region of lower limb muscle representation of the motor cortex. The coil orientation was adjusted to deliver counter-clockwise current flow in the left hemisphere and clockwise current flow in the right hemisphere. The stimulations were initiated manually every 6 to 9 s in a pseudorandom fashion to avoid anticipation. For the main trials, the stimulation intensity was set to 120% MT intensity for the testing pulse and to 80% MT intensity for the conditioning pulse. Two event channels connected to the trigger outputs of the Magstim stimulators were recorded simultaneously with the rest of the data to mark the time position of the TMS pulses generated (Fig.2).

Data analysis

Data analysis was performed using custom written scripts developed in Spike2 ver.4.15 analysis software (CED, Cambridge, UK).

Measures of cortical and corticospinal excitability included MEP latency, amplitude and total MEP area, as well as their inhibition (SICI) or facilitation (ICF) induced by paired stimulation. MEP amplitudes were measured peak-to-peak; MEP latencies were measured between the end of the TMS stimulus and the beginning of the MEP; MEP total area was calculated from the rectified EMG signal between the start and the end of the MEP (Fig.2). Five single and five paired MEP's were recorded during each period of the trials. The parameters of each paired pulse MEP were expressed as a ratio to the average raw value of the corresponding parameter for the single pulse MEP recorded during the same period of the trial.

The level of pre-stimulation EMG muscle activity was assessed by calculating the total area of the rectified EMG signal in the 500ms preceding the delivery of each TMS pulse

(Fig.2). The kinematic effect of each TMS was quantified by the change in the knee flexion angle following the stimulation (Fig.2). The average parameter values were calculated for each condition (with- and without-vibration WBV), period of squat (pre-, during, and post-WBV) and type of TMS regime (single and paired) and compared for statistical differences.

Spectral analysis of the EMG data recorded during a 5-s segment before the first TMS delivered during each exercise period was performed by Fast Fourier Transformation (FFT) with a block size of 2.048s using a Hanning window function and presented between 0 and 1000Hz in 2048 bins at a resolution of 0.4883Hz. Special care was taken during the experiments to minimize the contamination of the EMG signal with movement artefacts. The skin under the electrodes was carefully cleaned to reduce the skin impedance. The EMG electrodes were firmly attached to the skin with special double-sided medical tape. Also the electrode cables were twisted around each other, additionally shielded and affixed to the leg at multiple points. Despite these precautions, high energy peaks at the fundamental vibration frequency (30Hz) and harmonics (60, 90, 120 Hz) were present in the power spectrum of the EMG signal recorded during the second squat period (Fig.3A) in all WBV trials. These artefacts were absent from the first and third squat periods in the same trials where the vibration platform was switched off. Abercromby *et al.*, (2007) also observed excessive power of the EMG signal at vibration frequencies and their harmonics which they attributed (at least the dominant part of it) to the current induced in the electrode and the cables by the motion of the vibrating platform. In order to eliminate these motion artefacts at the dominant and the secondary harmonic vibration frequencies a combination of smoothing and filtering procedures was developed (adapted from Mewett *et al.*, 2004). The procedure is based on the assumption that the signal represents a mixture of sinusoids of different frequencies and amplitudes. In brief, data were subdivided into blocks of one period of the sinusoidal waveform to be removed. The wave amplitude and phase in each block were determined by

multiplying the source data by a sine and a cosine wave of the removed frequency, which was then subtracted from the original signal on a cycle by cycle basis. Before subtraction the amplitude of the removed sinusoid was corrected by a ratio calculated from the power spectral density of the signal to reflect the proportion of the signal power at the removed frequency above the average power of 2 neighbouring frequencies on each side of the spectrum. This procedure was performed for 30Hz and any harmonic frequencies that were present in the signal, and applied to the EMG records from all muscles and trials (with- and without- WBV). Comparison of the power spectral density before and after the 'spectral smoothing' procedure indicated that the vibration induced artefacts were successfully removed without excessive loss of signal power which usually happens when using notch digital filters (Fig.3A). The filtering procedure employed in this study (at 30, 60, 90 and 120 Hz) was unlikely to skew the parameters measured from the evoked potentials. We have directly demonstrated this by comparing MEP parameters on filtered and unfiltered data sets (Fig.3B)

Statistical analysis

Due to the experimental design, MEP parameters in response to single pulse TMS were available from two visits [2 control trials (SICI and ICF); 2 vibration trials (SICI and ICF)]. Therefore initially, a three-factor repeated-measures ANOVA [repeat (2 visits); condition (2 levels: with- and without WBV); squat period (3 levels: before-, during-, after- WBV)] was used to test for the main and interaction effects of experimental parameters on MEP parameters in response to single pulse TMS. However, there were no significant main or interaction effects involving the factor 'repeat' and therefore the average parameter values from the two visits were calculated. These averaged data and the MEP parameters in response to paired pulse TMS with ISI=13ms (ICF), and with ISI=3ms (SICI) were analysed by two-way repeated

measures ANOVA (condition vs squat period). When significant condition vs squat period interaction effects were established, the percentage differences between parameter values in the second and third squat periods to the first squat period were calculated and statistically compared between conditions using post-hoc paired Student's t-tests corrected for multiple comparisons using Holm-Sidak step-down procedures.

The reliability of MEP and kinematic measures in response to single and paired TMS stimulation was evaluated using the data from the first squat period of each of 4 completed trials. The reliability assessment was based on intra-class correlation analysis using a one-way random-effects average measure model (1,1) to calculate the intraclass correlation coefficients (ICC). The overall acceptable significance level of differences for all statistical tests was set at $p < 0.05$. All statistical analyses were performed in SPSS for Windows version 13 (SPSS Inc., Chicago, IL) and Origin version 6.0 (Microcal Software Inc.) package software. For descriptive purposes percentage differences between the conditions and the squat periods were calculated.

Results

The ICC values for the analysed parameters range from 0.58 (TA MEP latency during SICI protocol) to 0.98 (TA MEP amplitude during single TMS) indicating fair-to-good repeatability of the measures employed in the current study.

Responses to single pulse TMS

TA muscle. The TA MEP peak-to-peak amplitude and MEP total area demonstrated a significant condition vs period interaction effect ($p = 0.003$ and $p = 0.035$, respectively) as well as significant main effect of squat period ($p < 0.0001$ for both, Fig.4A). During vibration

exposure TA MEP amplitude ($56\pm 14\%$ vs $11\pm 5\%$, $p=0.031$, vibration vs control trial) and TA MEP total area ($71\pm 19\%$ vs $13\pm 8\%$, $p=0.022$, vibration vs control trial) were increased to a significantly greater degree during the second period relative to first period of squat exercise. In the WBV compared to the control trials both TA MEP parameters remained elevated during the third (post-vibration) period but this did not attain statistical significance between conditions (amplitude: $23\pm 10\%$ vs $17\pm 6\%$, $p=0.518$; area: $32\pm 11\%$ vs $18\pm 8\%$, $p=0.140$, increase during third period relative to first period of squat exercise; vibration vs control). There were no significant effects on the latency of the TA MEPs (condition: $p=0.529$; squat period: $p=0.779$; interaction: $p=0.973$) or on the pre-stimulation level of EMG activity (condition: $p=0.871$; squat period: $p=0.128$; interaction: $p=0.645$) observed in any condition or squat period (Fig.4A). Examples of the MEPs recorded in TA muscle in response to single pulse TMS are presented in Fig.5.

SOL muscle. There was a significant main effect of squat period on both SOL MEP peak-to-peak amplitude and area ($p=0.002$ and $p=0.014$, respectively, Fig.4B), but there was no significant condition ($p=0.188$ and $p=0.363$, respectively) or interaction effect ($p=0.117$ and $p=0.103$, respectively). SOL pre-stimulation EMG activity was not significantly different (condition: $p=0.354$; squat period: $p=0.289$; interaction: $p=0.608$) between conditions or squat periods. Nor was there any effect of condition or squat period on the latency of the SOL MEPs (condition: $p=0.244$; squat period: $p=0.129$; interaction: $p=0.952$; Fig.4B).

Responses to paired pulse TMS

Short-latency intracortical inhibition (SICI). For SOL MEP amplitude ($p=0.027$) and area ($p=0.019$), and TA MEP amplitude ($p=0.009$) there were significant condition vs squat period interaction effects (Fig.6A,B). In vibration trials, the values of the MEP parameters of both muscles were lower during vibration exposure (2nd squat period) compared to the 1st non-

vibration period (amplitude: $-19\pm 4\%$, $p=0.007$ and $-13\pm 4\%$, $p=0.031$; total area: $-19\pm 8\%$, $p=0.030$ and $-17\pm 6\%$, $p=0.035$; in TA and SOL respectively) showing significantly increased intracortical inhibition during vibration. In the vibration trials, MEP parameter values in SOL continued to decline during post-vibration squat period (amplitude to $-22\pm 6\%$, $p=0.021$ and total area to $-28\pm 6\%$, $p=0.006$ decrease relative to first squat period), whereas in TA muscle the MEP parameters returned to values similar to those observed pre-vibration (amplitude difference of $-1\pm 4\%$, $p=0.781$ and total area of $-9\pm 6\%$, $p=0.261$). There was no effect of condition or squat period on the latency of the MEPs recorded in both TA (condition: $p=0.230$; squat period: $p=0.113$; interaction: $p=0.330$) and SOL (condition: $p=0.357$; squat period: $p=0.726$; interaction: $p=0.487$; Fig.6 A,B). Pre-paired stimulation EMG activity was not significantly different between conditions or squat periods (TA: condition: $p=0.449$, squat period: $p=0.317$, interaction: $p=0.604$; SOL: condition: $p=0.529$, squat period: $p=0.108$, interaction: $p=0.103$; Fig.6C).

Intracortical facilitation (ICF). There was a main effect of squat period for both TA MEP peak-to-peak amplitude and MEP total area ($p=0.010$ and $p=0.049$ respectively, Fig.7A). In addition there was a significant condition vs squat period interaction effect for TA MEP amplitude ($p=0.036$) and a similar pattern of change was observed for TA MEP area but this did not achieve statistical significance ($p=0.162$). Intracortical facilitation (TA MEP amplitude) decreased to a greater extent over the squat periods in the vibration than in the control trials ($-21\pm 4\%$ vs $-3\pm 4\%$ change during second versus first squat period, $p=0.026$; vibration vs control). TA MEP latency was not affected by squat period ($p=0.543$) or condition ($p=0.225$) or condition vs squat period interaction ($p=0.742$). There were no significant effects of any of the studied factors (condition or squat period) on any SOL MEP parameter ((amplitude: condition: $p=0.591$, squat period: $p=0.816$, interaction: $p=0.388$; total area: condition: $p=0.781$, squat period: $p=0.990$, interaction: $p=0.452$; latency: condition: $p=0.838$, squat period: $p=0.518$,

interaction: $p=0.551$; Fig.7B). Pre-paired stimulation EMG activity was not significantly different between conditions or squat periods (TA: condition: $p=0.989$, squat period: $p=0.112$, interaction: $p=0.224$; SOL: condition: $p=0.490$, squat period: $p=0.967$, interaction: $p=0.665$; Fig. 7C).

Knee joint angle changes

The average knee flexion angle at the time of TMS delivery was not significantly different between the trials, conditions and squat periods (SICI trials: $34.5\pm 1.6^{\circ}$ vs $34.8\pm 1.8^{\circ}$, $p=0.637$; ICF trials: $34.2\pm 1.3^{\circ}$ vs $34.1\pm 1.9^{\circ}$, $p=0.780$; control vs vibration). These values are slightly higher than the pre-set protocol value of 30° knee flexion since one of the subjects needed to assume a deeper squat (40°) position in order to diminish transmission of the vibration to the head. Knee flexion angle was kept constant throughout each subject's 4 trials. Knee flexion angle decreased in response to both single and paired pulse TMS (Fig.8A,B). In comparison to static squat alone, the decrease in knee flexion angle tended to be smaller ($p=0.061$, condition vs squat period interaction) in response to single pulse TMS during vibration. In response to paired pulse with ISI of 3ms the decrease in knee flexion angle was larger during vibration than during static squat alone ($p=0.015$, condition vs squat period interaction). In response to paired pulse with ISI of 13ms the decrease in knee flexion angle was not different between conditions ($p=0.806$) or squat periods ($p=0.641$) or their interaction ($p=0.293$). This pattern of change is reciprocal to the vibration-induced changes in MEP amplitude and area, i.e. MEP amplitude and area were smaller where the decrease in knee flexion angle was amplified and vice versa.

Discussion

To our knowledge, this is the first study to determine the effects of low frequency whole body vibration during exercise on corticospinal excitability in parallel with kinematic changes (knee joint angle changes). The key findings of this study are: 1) WBV applied during static squat exercise increased TA corticospinal pathway excitability (higher TA MEP amplitude and total area in response to single pulse suprathreshold TMS); 2) vibrated squat exercise increased intracortical inhibition of the neurons related to the activation of both SOL and TA muscles; 3) a significant reduction in the intracortical facilitatory processes related to TA muscle activation was observed during vibrated squat exercise; 4) knee joint angle changes occurred in parallel with altered TA and SOL corticospinal pathway excitability. These data suggest that acute exposure (110s) to 30Hz 1.5mm WBV during static squat increased the excitability of the corticospinal pathways related to the TA muscle activity relative to static squatting exercise without vibration. In parallel increased intracortical inhibition and decreased intracortical facilitation were observed. Therefore, this study for the first time demonstrates that the effects of WBV are not entirely restricted to the periphery but also involve corticospinal and intracortical processes. This exciting potential for WBV to modulate cortical plasticity requires further investigation. In the present experiment no significant changes in the excitability of SOL corticospinal pathways in response to single pulse TMS or in the intracortical facilitatory processes related to SOL muscle activation were observed during vibrated compared to non-vibrated squat exercise. This could be related to the functional differences between the two muscles, differences in their pre-activation level, differences in the strength of corticospinal projections to TA and SOL motor neurones (Perez *et al.*, 2004), that TMS stimulation intensity was optimised for TA not SOL motor threshold or that sample size power calculations were based on TA MEP responses.

Cardinale & Lim (2003) found that the root-mean-square amplitude of vastus lateralis EMG activity was higher during vibration in the 30-40Hz range than 50Hz. Therefore in the

present study we elected to expose subjects to 30Hz, low amplitude (1.5mm) vibration of 110 s duration during a static semi-squat. Significantly greater transmission of the vibration (g-forces) during vertical sinusoidal WBV has been found with semi-squat than standing postures (Crewther *et al.* 2004). For vastus lateralis, gastrocnemius and tibialis anterior the magnitude of the neuromuscular response to vertical WBV was shown to be greatest at smaller (below 30⁰) knee flexion angles (Abercromby *et al.* 2007). Therefore, knee flexion angle of 30⁰ was selected to limit vibration transmission to the head which induces visual disturbance and nausea.

In the present study we for the first time demonstrate that low frequency whole body vibration superimposed during static squat exercise increased the amplitude of MEPs in TA but not SOL. SICl was increased in both TA and SOL muscles during vibration and this effect was still present in SOL after cessation of the vibration exposure. High frequency vibration also augments motor cortex excitability (Siggelkow *et al.* 1999; Kossev *et al.* 2001). However in contrast to the effects of whole body low frequency vibration presented here, targeted high frequency vibration of the muscle or tendon has been shown to reduce short-interval intracortical inhibition (Rosenkranz & Rothwell, 2006), whilst the opposite occurs within neighbouring and contralateral muscles (Siggelkow *et al.* 1999; Rosenkranz & Rothwell, 2003). There are a number of factors that may help to explain the discrepancies between the present findings and those of HFV studies: 1) vibration frequency *per se*; 2) whole body vs targeted muscle or tendon vibration 3) stimulation of lower rather than upper limb muscles.

Microneurographic recordings in healthy humans have shown that low-amplitude (0.2–0.5mm) muscle tendon vibration of a relaxed muscle is a powerful and selective stimulus of activity in Ia afferents by entraining the discharge rate of primary muscle spindle endings (Roll *et al.* 1989). The Ia afferent firing rate is entrained linearly with vibration frequencies up to 70–80Hz, followed by a subharmonic increase at higher frequencies, with sharp falls often

observed at frequencies between 150 and 200Hz (Roll *et al.* 1989). It is therefore perhaps not unexpected that there are differences between the effects observed in the present study and those induced by high frequency vibration. Certainly the apparent beneficial effect of chronic low frequency vibration differs from the detrimental neurological symptoms such as white finger induced by chronic exposure to high frequency vibration.

Experimentally, high frequency vibration is introduced by direct muscle or tendon stimulation whereas WBV activates the proprioceptive input of all antagonist/synergist muscles and acts simultaneously on the motor and sensory afferents of all limb muscles. WBV induces sensory stimulation of foot-sole afferents as well, which are well known to play an important role in postural control (Bruyere *et al.* 2005).

The majority of published studies examining the effects of high frequency vibration have been conducted in upper limb muscles, primarily elbow flexors or hand muscles. Whereas, in the present study due to the damping of vibration during its passage through the body we elected to interrogate muscles close to the vibrating platform i.e. shank muscles and TA in particular. However, there is a decline in the strength of corticomotoneuronal connections from upper to lower limb muscles (Brouwer & Ashby, 1990) which may account in part for the apparent differences between the effects of high frequency vibration and those observed in the present study.

During WBV squat exercise TA exhibited increased MEP alongside increased SICI and decreased ICF whereas in SOL only intracortical inhibition of the neurons related to the muscle activation was increased. These muscle specific responses may be related to differences in their function (dorsi vs plantar flexion) or pre-activation level. However, we cannot confirm the latter since SOL and TA pre-activation EMG levels were not normalised to maximal activation and are therefore not comparable. In addition, the corticospinal projections to TA motoneurons are much stronger than for other leg muscles and may even be of the

same magnitude as for the hand muscles (Perez *et al.* 2004). Differences in the effects of WBV on the corticospinal pathway and intracortical circuitry of TA and SOL might therefore be expected. However we cannot rule out that the differences in the responses in TA and SOL are due to sub-optimal TMS pulse intensity for SOL and low statistical power.

Similar positioning of corticomotoneuronal synapses onto the SOL and TA populations of motoneurons has been demonstrated (1.13 vs 1.14ms rise time of monosynaptic EPSPs in TA and SOL; de Noordhout *et al.* 1999), however of all muscles, tested with transcranial electric stimulation, the responses were smallest in SOL. Therefore, SOL requires a stronger stimulus intensity to produce a response. In the present study the intensity of the TMS was adjusted to be suprathreshold for TA (120% MT for TA), which may not be the optimal stimulation intensity for activation of the SOL corticospinal projections, certainly SOL MEPs were on average 30% smaller than in TA.

As observed in previous studies (Bawa *et al.* 2002), there was a higher degree of variability between subjects in the SOL than TA responses. Four subjects from the studied population demonstrated a clear increase in SOL MEP during the WBV compared to the control; MEP responses were similar between conditions for the other two subjects and 1 subject responded with higher SOL MEP to single pulse TMS in the control than in WBV trials. This high degree of variability in SOL MEP excitability in response to WBV may be related to variation in the postural strategies adopted by subjects to maintain their balance in the semi-squat posture on the vibration platform and/or inconsistent afferent stimulation across subjects. The observed changes in SOL MEPs although not as strong as those in TA could be in response to disturbance of the postural balance during WBV. The subjects were instructed to concentrate on keeping their knee flexion angle constant (visual feed-back provided on a monitor) and compensate for the disturbance induced by the TMS, however it was visible that some were able to do that more easily and effectively than others. Thus,

different attention level may be another factor for the observed differences especially when sensory stimulation is used in the intervention protocol (Rosenkrantz & Rothwell, 2006).

In our hands, WBV had a complex effect on corticospinal pathway excitability: increased MEPs, increased SICI and decreased ICF. MEP amplitude depends on the excitability of synaptic relays in the corticospinal connections at both cortical and spinal level (Devanne *et al.* 1997). Whereas, paired pulse TMS is thought to test the excitability of intrinsic GABAergic inhibitory and facilitatory circuits in the motor cortex (Ziemann *et al.* 1996); which converge onto the cortical motor neurons and affect their excitability (Kossev *et al.* 2003). It is however plausible that MEP amplitude can increase despite reduced facilitation and increased intracortical inhibition: first, intracortical and corticospinal pathways represent different neuronal circuits which can therefore be influenced independently (Stefan *et al.* 2002); and secondly the increase in corticospinal pathway excitability may be primarily related to changes at the spinal level. Muscle afferent feedback is of fundamental importance for motor plasticity, especially for the muscles of the lower limb (Hulliger, 1993). Previously Rosenkrantz & Rothwell (2006) have shown that different plasticity protocols (namely motor practice, direct high frequency muscle vibration and paired associative stimulation) can independently manipulate MEP amplitude, SICI and sensorimotor organisation in specific ways. In conclusion, whole body vibration during exercise was associated with increased corticospinal excitability and alteration of intracortical processes (increased intracortical inhibition and decreased facilitation) relative to exercise alone. These findings suggest that low frequency whole body vibration has the potential to induce motor plasticity and highlights the need for future research into the neural mechanisms of WBV physiological effects.

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Figure legends

Figure 1. Experimental protocol.

Figure 2. Representative data from a single subject showing the motor evoked potentials (MEPs) elicited by a single pulse transcranial magnetic stimulation (registered as an event on channel 'stim B') in tibialis anterior (TA) and soleus (SOL) muscles and the change induced in the depth of static squat (knee joint flexion angle).

Figure 3. A - Example of the power spectral density calculated from the EMG signal recorded from soleus (SOL) muscle during static squat exercise with whole body vibration (30Hz, 1.5mm) before (unfiltered, grey line) and after (filtered, black line) removal of the vibration artefacts by 'spectral smoothing' method. **B** - Removal of the vibration artefacts by 'spectral smoothing' method does not affect the time and amplitude parameters of the motor evoked potentials (MEPs) recorded in the tibialis anterior (TA) and soleus (SOL) muscles.

Figure 4. Average population (mean \pm SEM, n=7) values of the motor evoked potential (MEP) parameters calculated from the responses to single pulse TMS recorded from the tibialis anterior (**A**) and soleus (**B**) muscles during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); (* - main squat period effect; \otimes - condition vs period interaction effect; p<0.05).

Figure 5. Example of the motor evoked potentials (MEPs) recorded from tibialis anterior (TA) muscle in response to single pulse transcranial magnetic stimulation during static squat

exercise performed without- (control trial) or with- (vibration trial) whole-body vibration during the second squat period (WBV, +/- vib).

Figure 6. Average population (mean \pm SEM, n=7) values of motor evoked potential (MEP) parameters calculated from the responses to paired pulse transcranial magnetic stimulation (TMS) with interstimulus interval (ISI) of 3ms recorded from the tibialis anterior (**A**) and soleus (**B**) muscles during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); **C** – average level of the pre-stimulation TA and SOL EMG activity; (* - main squat period effect; \otimes - condition vs period interaction effect; p<0.05).

Figure 7. Average population (mean \pm SEM, n=7) values of the motor evoked potential (MEP) parameters calculated from the responses to paired pulse transcranial magnetic stimulation (TMS) with interstimulus interval (ISI) of 13 ms recorded from the tibialis anterior (**A**) and soleus (**B**) muscles during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); **C** – average level of the pre-stimulation TA and SOL EMG activity; (* - main squat period effect; \otimes - condition vs period interaction effect; p<0.05).

Figure 8. A - Population average (mean \pm SEM, n=7) decrease in the knee flexion angle in response to single and paired pulse transcranial magnetic stimulation (TMS) during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); **B** – Example of the knee flexion angle changes in response to: **left panel** – single pulse TMS pre-whole-body vibration (pre-

WBV, grey line), during WBV (black line), and post-WBV (light grey line) in a vibration trial; **middle panel** - paired TMS with interstimulus interval (ISI) of 3ms (short-latency cortical inhibition, SICl) during the second period of squat exercise with- (black line) and without- (grey line) whole body vibration; **right panel** – ISI of 13ms (intracortical facilitation, ICF) during second period of squat exercise with- (black line) and without- (grey line) whole body vibration; the vertical arrow in each panel marks the time point of TMS pulse delivery.

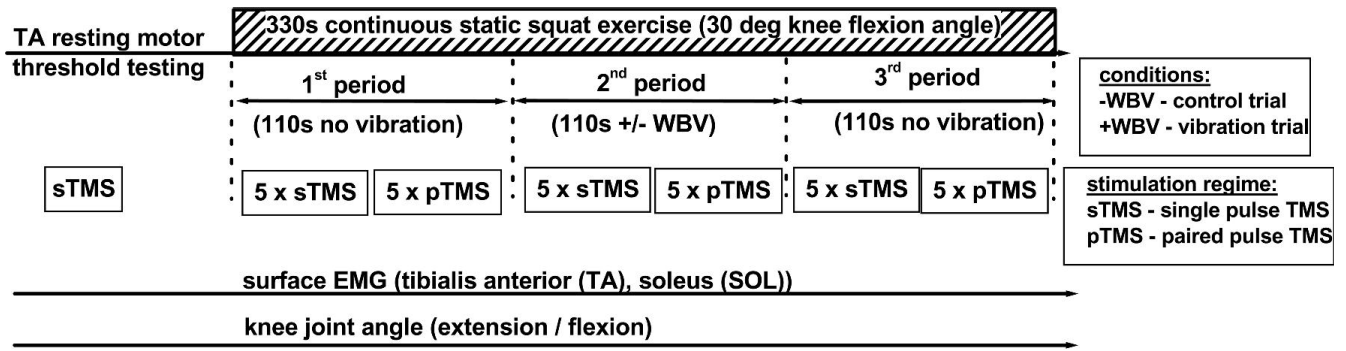


Figure 1. Experimental protocol.

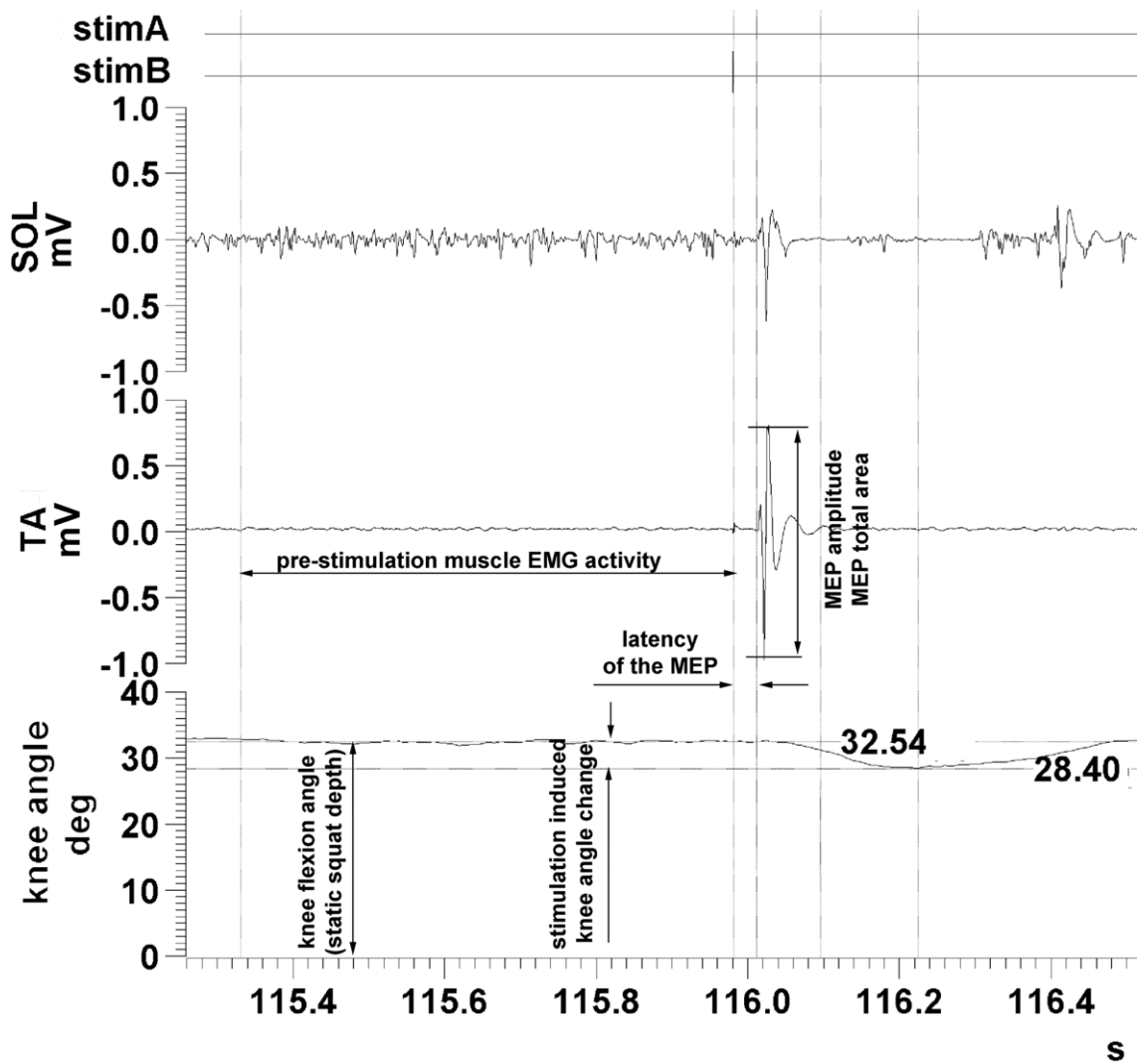
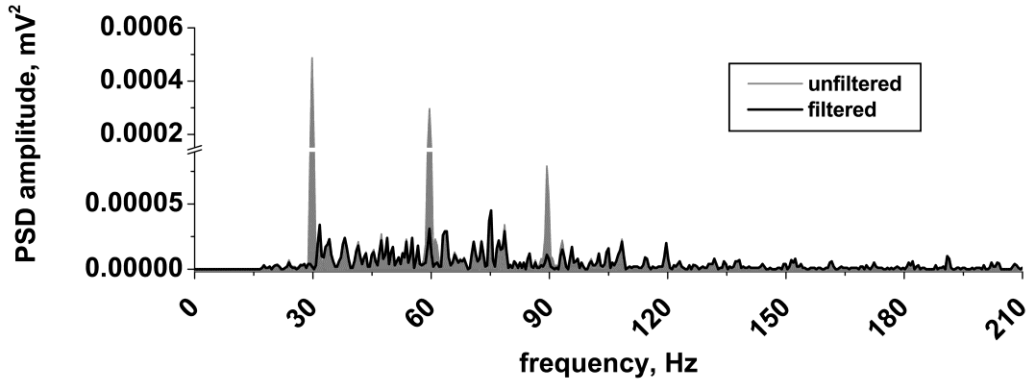


Figure 2. Representative data from a single subject showing the motor evoked potentials (MEPs) elicited by a single pulse transcranial magnetic stimulation (registered as an event on channel 'stim B') in tibialis anterior (TA) and soleus (SOL) muscles and the change induced in the depth of static squat (knee joint flexion angle).

A. EMG power spectrum density in SOL



B. Effect of EMG filtering on MEPs

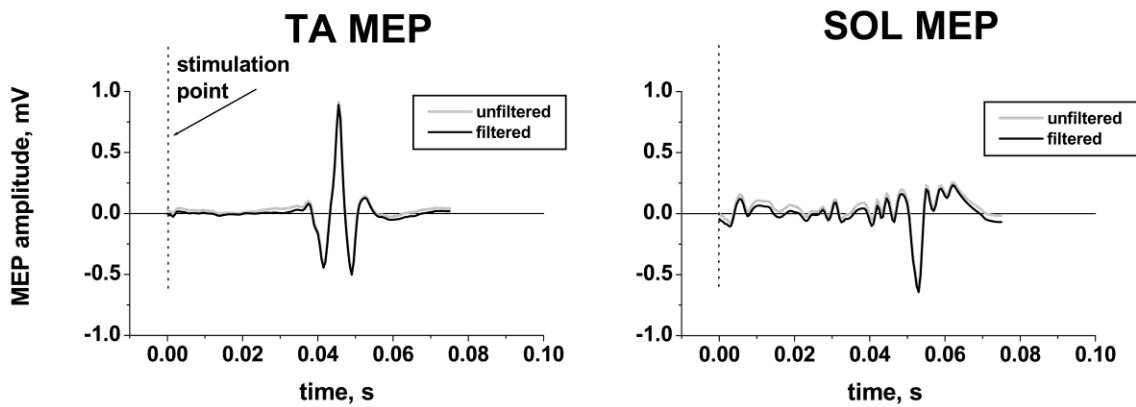


Figure 3. A - Example of the power spectral density calculated from the EMG signal recorded from soleus (SOL) muscle during static squat exercise with whole body vibration (30Hz, 1.5mm) before (unfiltered, grey line) and after (filtered, black line) removal of the vibration artefacts by 'spectral smoothing' method.

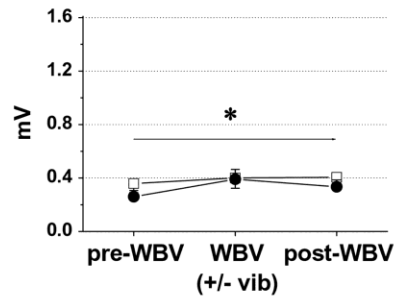
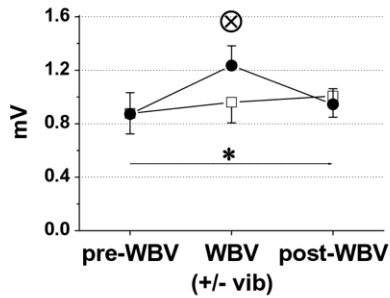
B - Removal of the vibration artefacts by 'spectral smoothing' method does not affect the time and amplitude parameters of the motor evoked potentials (MEPs) recorded in the tibialis anterior (TA) and soleus (SOL) muscles.

Single pulse TMS regime

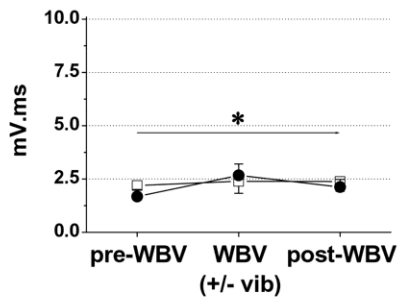
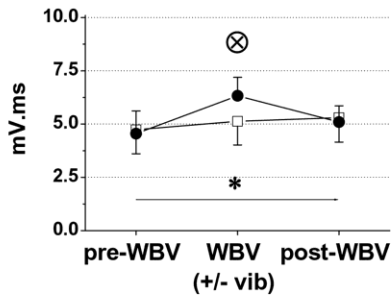
A. Tibialis anterior (TA)

B. Soleus (SOL)

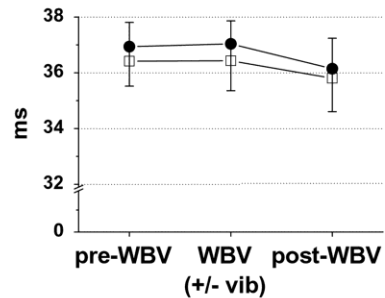
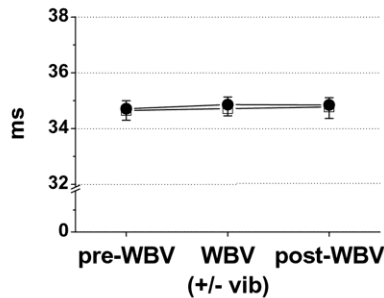
MEP amplitude



MEP total area



MEP latency



pre-TMS EMG level

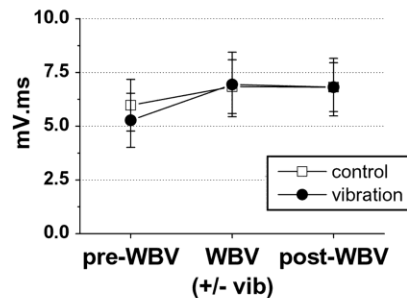
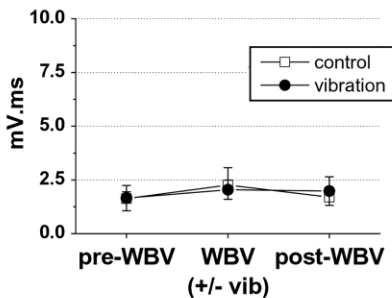


Figure 4. Average population (mean \pm SEM, n=7) values of the motor evoked potential (MEP) parameters calculated from the responses to single pulse TMS recorded from the tibialis anterior (A) and soleus (B) muscles during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); (* - main squat period effect; \otimes - condition vs period interaction effect; $p < 0.05$).

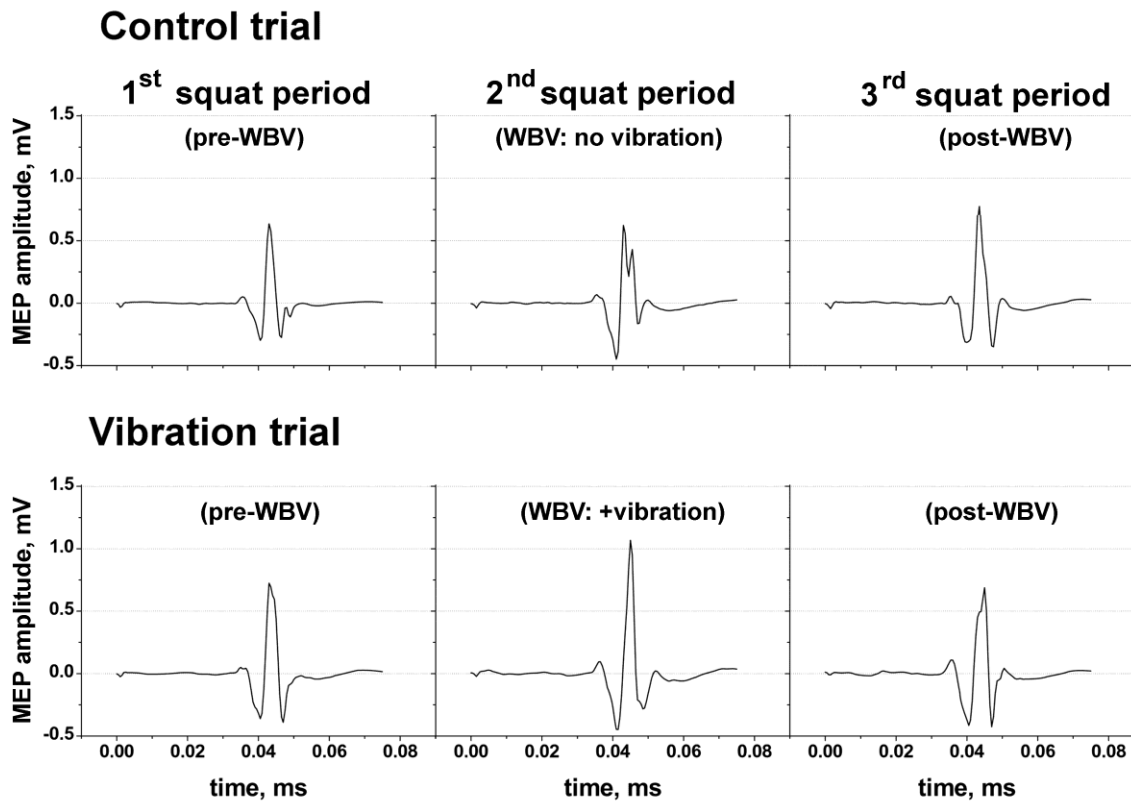


Figure 5. Example of the motor evoked potentials (MEPs) recorded from tibialis anterior (TA) muscle in response to single pulse transcranial magnetic stimulation during static squat exercise performed without- (control trial) or with- (vibration trial) whole-body vibration during the second squat period (WBV, +/- vib).

Paired pulse TMS regime (ISI = 3 ms)

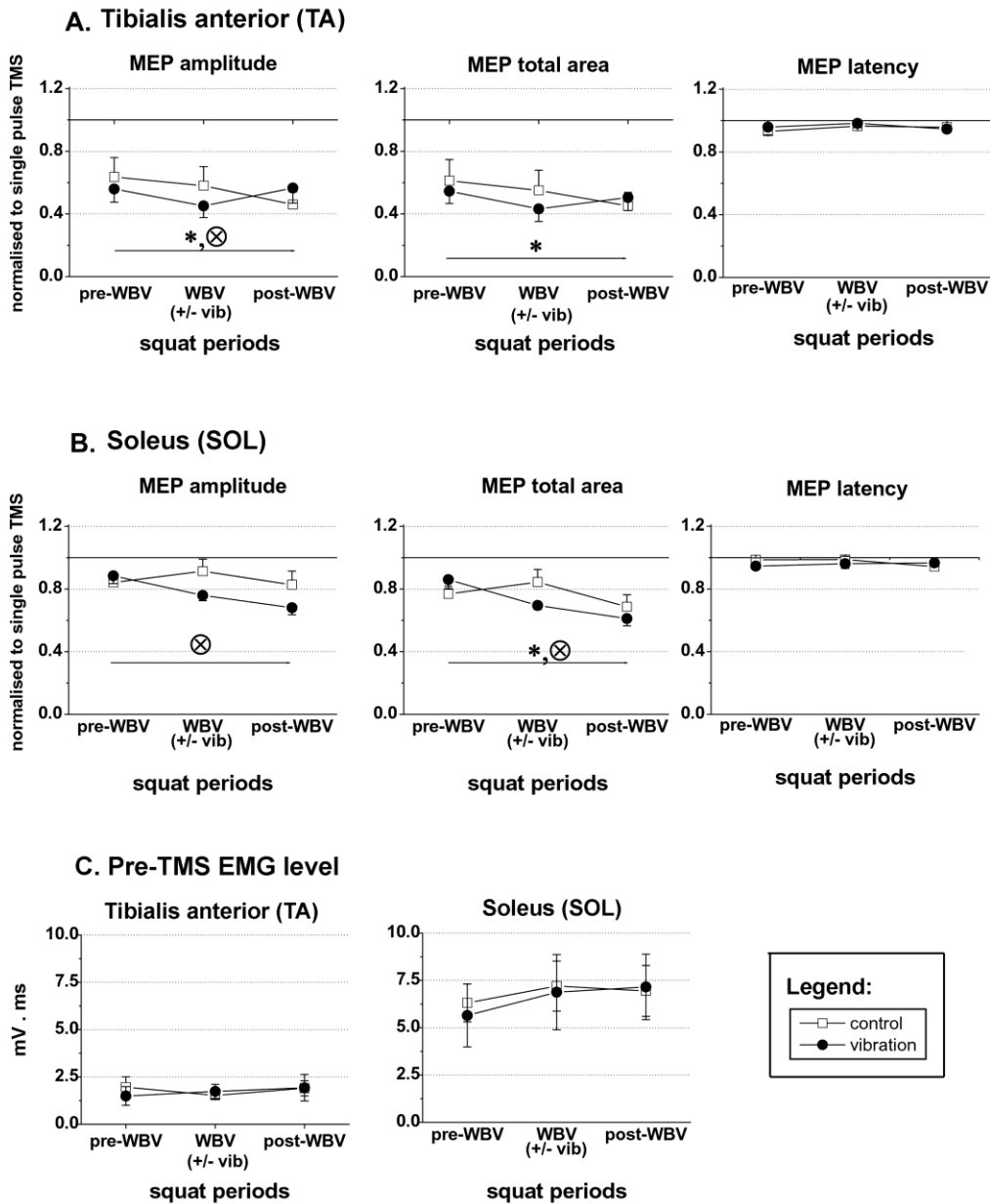


Figure 6. Average population (mean±SEM, n=7) values of motor evoked potential (MEP) parameters calculated from the responses to paired pulse transcranial magnetic stimulation (TMS) with interstimulus interval (ISI) of 3ms recorded from the tibialis anterior (A) and soleus (B) muscles during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); C – average level of the pre-stimulation TA and SOL EMG activity; (* - main squat period effect; ⊗ - condition vs period interaction effect; p<0.05).

Paired pulse TMS regime (ISI = 13 ms)

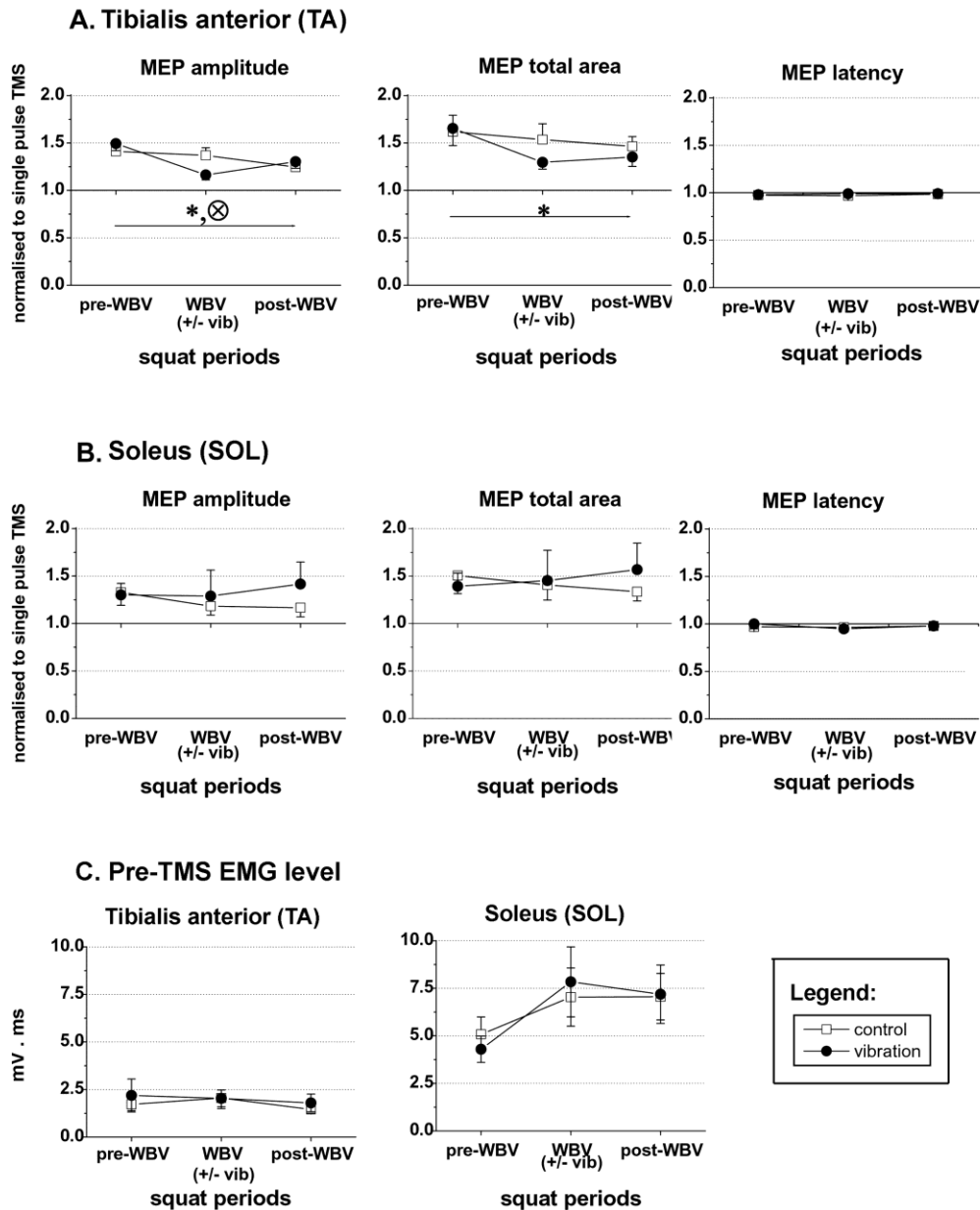
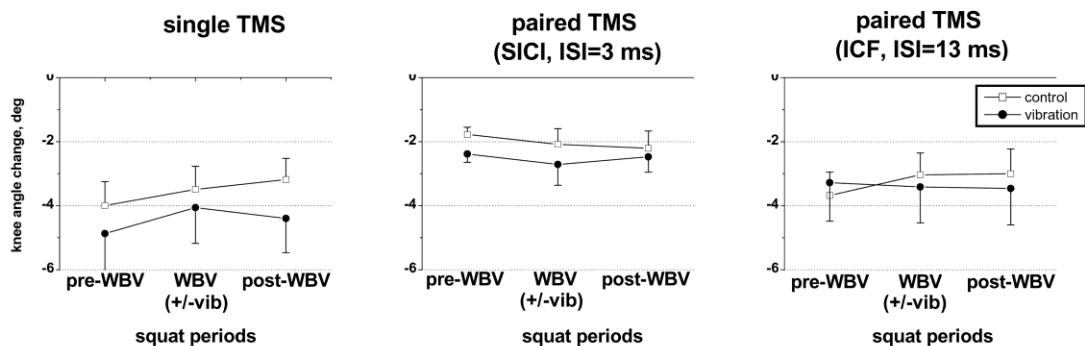


Figure 7. Average population (mean \pm SEM, n=7) values of the motor evoked potential (MEP) parameters calculated from the responses to paired pulse transcranial magnetic stimulation (TMS) with interstimulus interval (ISI) of 13 ms recorded from the tibialis anterior (**A**) and soleus (**B**) muscles during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib); **C** – average level of the pre-stimulation TA and SOL EMG activity; (* - main squat period effect; \otimes - condition vs period interaction effect; $p < 0.05$).

A. Average knee flexion angle changes



B. Example of knee flexion angle changes

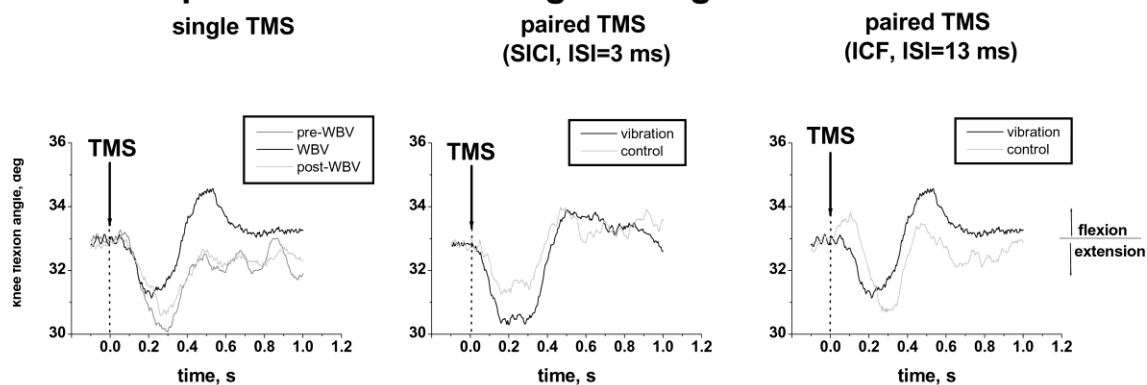


Figure 8. A - Population average (mean \pm SEM, n=7) decrease in the knee flexion angle in response to single and paired pulse transcranial magnetic stimulation (TMS) during static squat exercise performed with- (black circles) or without- (transparent squares) whole-body vibration during the second squat period (WBV, +/- vib);

B - Example of the knee flexion angle changes in response to: **left panel** - single pulse TMS pre-whole-body vibration (pre-WBV, grey line), during WBV (black line), and post-WBV (light grey line) in a vibration trial; **middle panel** - paired TMS with interstimulus interval (ISI) of 3ms (short-latency cortical inhibition, SICI) during the second period of squat exercise with- (black line) and without- (grey line) whole body vibration; **right panel** - ISI of 13ms (intracortical facilitation, ICF) during second period of squat exercise with- (black line) and without- (grey line) whole body vibration; the vertical arrow in each panel marks the time point of TMS pulse delivery.