Rieder, F., Wiesinger, H. P., Kösters, A., Müller, E., Seynnes, O. R. (2015). Whole-body vibration training induces hypertrophy of the human patellar tendon. *Scandinavian Journal of Medicine & Science in Sports*, under utgivelse. doi:10.1111/sms.12522

Whole-Body Vibration Training Induces Hypertrophy Of The Human Patellar Tendon.

Rieder, F.[1], *Wiesinger, H.-P.*[1], *Kösters, A.*[1], *Müller, E.*[1], *Seynnes, O. R.*[2]

[1]: Department of Sport Science and Kinesiology, University of Salzburg, Salzburg, Austria

[2]: Norwegian School of Sport Sciences, Oslo, Norway

Running head: Tendon adaptations to vibration training

Address correspondence to:

Rieder Florian, MSc

Department of Sport Science and Kinesiology,

University of Salzburg,

Schlossallee 49

5400 Hallein/Rif

Austria

Phone: +43 662 8044 4883

Mail: florian.rieder@sbg.ac.at

Abstract

Animal studies suggest that regular exposure to whole-body vibration (WBV) induces an anabolic response in bone and tendon. However, the effects of this type of intervention on human tendon properties and its influence on the muscle-tendon unit function have never been investigated.

The aim of this study was to investigate the effect of WBV training on the patellar tendon mechanical, material and morphological properties, the quadriceps muscle architecture and the knee extension torque-angle relationship.

Fifty-five subjects were randomized into either a vibration, an active control or an inactive control group. The active control subjects performed isometric squats on a vibration platform without vibration. Muscle and tendon properties were measured using ultrasonography and dynamometry.

Vibration training induced an increase in proximal (6.3%) and mean (3.8%) tendon cross-sectional area, without any appreciable change in tendon stiffness and modulus or in muscle architectural parameters. Isometric torque at a knee angle of 90° increased in active controls (6.7%) only and the torque-angle relation remained globally unchanged in all groups.

The present protocol did not appreciably alter knee extension torque production or the musculotendinous parameters underpinning this function. Nonetheless, this study shows for the first time that WBV elicits tendon hypertrophy in humans.

Keywords: Mechanical properties, material properties, strength, length-tension relationship

Introduction

Vibration exposure, and in particular whole body vibration (WBV), has become a very popular training method in the last fifteen years. Chronic exposure to this type of loading has been shown to increase lower limb muscle strength (Torvinen et al., 2002; Colson et al., 2010), muscle mass (Gilsanz et al., 2006; Bogaerts et al., 2007), or jump performance (Torvinen et al., 2002; Colson et al., 2010). However, a few authors did not find any change in these parameters after WBV training (De Ruiter et al., 2003; Osawa et al., 2011). In fact, only a handful of publications indicate significant gains in lower limb muscle strength when protocols included an active control group performing the same exercises required for the subjects exposed to WBV (Delecluse et al., 2003; Osawa & Oguma, 2013). Some factors such as vibration parameters or the baseline physical fitness of the subjects may have mitigating effects but the cause of this inconsistency remains globally unknown. Interestingly, some authors have also reported a shift in the optimal joint angle of torque production after acute (Kemertzis et al., 2008; Pellegrini et al., 2010) and repeated (Savelberg et al., 2007) WBV exposure in a static position. For example, after four weeks of WBV training Savelberg et al. (2007) observed a ~8° shift of optimum knee angle towards extension for subjects training in an upright position and a ~4° shift towards a more flexed knee joint for subjects training in a squatting position. The authors suggested alterations in the number of sarcomeres in series as a possible explanation for these changes in the muscle force-length relation. Accordingly, the number of sarcomeres in series seems to be linked to the muscle operating length (Williams & Goldspink, 1973). Furthermore, it has been shown in animals (Williams & Goldspink, 1973) and humans (Blazevich et al., 2007; Reeves et al., 2009) that changes in fascicle length following immobilization and training can directly affect the muscle length-tension relationship. Such changes may also occur following WBV training and could explain why strength gains at a given joint angle may have gone undetected in some studies. However, to the best of our knowledge, no study has to date investigated the influence of WBV training on these parameters in healthy individuals.

Furthermore, theoretical models suggest that the mechanical properties of series elastic elements (e.g. tendons) can influence the force-length relationship of the whole muscle tendon unit (MTU): For a given MTU length, a change in tendon stiffness would induce a departure from optimal muscle length for force production (Lieber et al., 1992). Tendons are metabolically active structures (Bojsen-Møller et al., 2006), capable of morphological and structural plasticity following prolonged, increased loading (Couppé et al., 2008). Increases in stiffness and Young`s modulus (Kubo et al., 2002) and in some cases, increases in tendon cross sectional area (CSA) (Kongsgaard et al., 2007; Seynnes et al., 2009) are typically observed in the human Achilles and patellar tendon after various types of training interventions. Importantly, animal studies also indicate that prolonged exposure to vibration induces changes in collagen expression (Keller et al., 2013) and morphological/mechanical properties (Sandhu et al., 2011) of rat tendons. After five weeks of WBV training of rats, Sandhu et al. (2011) observed a 32% greater CSA and 41% greater stiffness of the flexor carpi ulnaris tendon compared to the control group. However, data regarding the influence of WBV on healthy human tendons and their possible functional impact are lacking. Such data would inform methodological approaches used to test muscular function following WBV training and extend our understanding of the effects of vibratory stimulus upon tendinous tissue.

Hence, the purpose of this single-blinded, randomized controlled study was i) to determine the effects of WBV training on the muscle architecture of the vastus lateralis muscle and on the mechanical properties of the patellar tendon and, ii) to investigate the possible influence of these adaptations upon the torque-angle relation of the quadriceps femoris MTU. We hypothesized that the high frequency cyclic loading imposed by the vibration would induce an increase in tendon stiffness and in VL fascicle length. Depending on the relative magnitude of changes in fascicle length and tendon stiffness, we expected that these changes could affect the torque-angle relationship in three possible directions: a) a shift in the optimal angle of torque production towards more extended knee joint angle (the increase in tendon stiffness exceeds the increase of fascicle length), b) a shift in optimal angle towards more flexed knee joint angle (the increase in fascicle length exceeds the increase in tendon stiffness) or c), no shift in optimal angle because changes in fascicle length and tendon properties cancel each other out.

Material and methods

Subjects

Fifty-five healthy adults (17 males and 38 females, age 32.3 ± 9.2 yr., height 1.72 ± 0.09 m, mass 71.5 ± 12.3 kg) with a sedentary lifestyle were recruited among students and employees of Salzburg University. Subjects were free of physical disabilities or orthopedic problems limiting testing of the right leg and had no prior experience of WBV training. Other exclusion criteria included pregnancy, diabetes, acute hernia, epilepsy, thrombosis and known cardiovascular disorders. All subjects were informed about the training and test protocols and signed a written declaration of consent. The study protocol was approved by the Ethics Committee of the University of Salzburg and conformed to the requirements of the Declaration of Helsinki.

Experimental design and training

A three-group intervention design was used to determine the effect of an 8-week WBV training program, while controlling for the effects of the exercise induced by the squatting position alone (active control group) and for the random variability of the measurements (inactive control group). Hence, subjects were randomly assigned to a whole-body vibration group (WBV, n = 19), an active control group (aCON, n = 20) and an inactive control group (iCON, n = 16). The subjects of the WBV- and aCON group trained on a vibration platform (Power Plate Next Generation, Power Plate ® GmbH, Frankfurt am Main, Germany) three times a week for an eight-week period. Each training session consisted in standing 10 times in a static squatting position for 60 seconds with a fixed knee angle of 50° (fully extended = 0°) and 60 seconds rest between repetitions. This knee angle was chosen after preliminary tests (n = 9), since it was suggestive of better transmission of vibration to the quadriceps muscle (as measured with a visual analogue scale) .In addition, visual analogue scale ratings indicated that flexion angles smaller than 50° led to more vibration being transmitted to the head, whereas vibration was stronger in the hip flexor muscles at angle greater than 50°. Finally, authors like Roelants et al. (2006) demonstrated that EMG of m. rectus femoris (RF) and m. vastus lateralis (VL) are equally elevated during WBV at knee joint angles of 55° and 90°, suggesting that a deeper squatting positions than the one chosen for this study would not induce further muscle activity. During training, the WBV group was exposed to a vertical sinusoidal vibration (frequency 30 Hz, amplitude 2 mm), whereas the aCON group performed identical exercises without vibration. With these settings, the peak acceleration as recorded with an accelerometer placed in the middle of the vibration platform (one-dimensional accelerometer biovision, Wehrheim, Germany) was about 2 g. The iCON subjects were instructed not to change their daily activities. Subjects were familiarized with the whole testing procedure one week prior to the start of the study. Post-tests were performed one week after the last training to avoid possible acute effects of the latest vibration session in the WBV group. Whilst tests performed earlier could have increased the probability to detect changes, a one-week delay was chosen because of the uncertainty regarding the time-course of fluid shifts in musculotendinous structures after WBV.

Muscle anatomical cross-sectional area

Cross-sectional area (CSA) of the right RF was measured with ultrasonography (LA523, 50 mm array, 10- to 15-MHz transducer, MyLab25, Esaote, Genoa, Italy). Subjects were lying in a supine position and muscle scans were taken at a distance corresponding to 40% of femur length (measured manually as the distance between the lateral femoral condyle and the trochanter major), relative to the lateral femoral condyle. At this location, the width of the RF did not exceed that of the ultrasound transducer.

Muscle architecture

Architecture of the right VL was assessed at rest with ultrasonography, at the same location as RF CSA along the femur length. Scanning positions of the transducer were recorded on acetate paper using moles and small angiomas as reference points to ensure consistency between measurements.

RF CSA, VL muscle thickness (Tm), pennation angle (ϴ) and fascicle length (Lf) were analyzed offline using a digitizing software (ImageJ 1.41, NIH, Bethesda, USA). Fascicles were segmented manually and portions running out of the scanning field of view were extrapolated as straight lines. The reliability of this approach has been shown previously (Muraoka et al., 2001). Pennation angle was defined as the angle between the deep aponeurosis and the fascicles. Owing to the limited ultrasound field of view, muscle thickness was measured as surrogate of CSA in the VL, as the distance between the deep and superficial aponeuroses. An average of three measurements was obtained for each parameter for the statistical analysis.

Tendon morphology and mechanical properties

Prior to testing, a warm-up procedure involved 8-minute cycling (Heinz Kettler GmbH and Co. KG, Ense-Parsit, Germany) at a submaximal intensity of 1.5 W/kg and a pedal rate of 70 rpm. Subjects were then seated on an isokinetic dynamometer (IsoMed 2000 D&R Ferstl GmbH, Hemau, Germany) with a hip angle of 60° (0° corresponding to supine position), a knee angle of 90° (0° corresponding to full extension) and strapped with safety belts. Patellar tendon length and CSA were measured in the longitudinal and the transversal planes, respectively, using ultrasonography. Tendon length was measured as the distance between the tibial insertion and the apex of the patella. Tendon CSA was measured proximally, below the apex of the patella (CSAp), at mid-length (CSAm) and distally, above the tibial insertion (CSAd). The same experimenter, blinded to the subjects' identity, analyzed all scans offline with ImageJ.

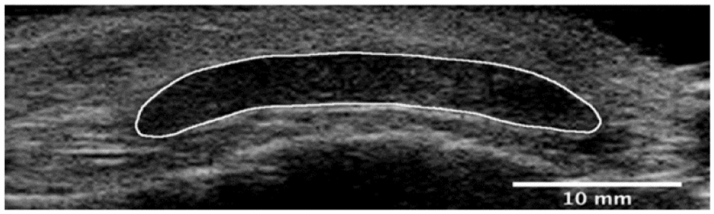

Figure1: Ultrasound scan above the insertion of the tibia showing the distal patellar tendon CSA.

To determine tendon stiffness and Young`s modulus, the patellar tendon elongation during isometric, ramped, maximal voluntary contractions was recorded with ultrasonography. A visual feedback was provided to the subjects, with the instruction to gradually increase knee extension torque at a constant loading rate (110 Nm/s; Kösters et al., 2014). Subjects were familiarized with this task with a minimum of three trials. Ultrasonographic data collection during contractions was synchronized with recordings of knee extension torque and electromyographic activity (EMG, see below). Tendon elongation was measured offline as the displacement of the patellar apex relative to the tibial plateau, using a software for semi-automated tracking (Tracker 4.8, Cabrillo.edu/-dbrown/tracker). The antagonistic co-activation torque during knee extension was calculated by assuming a linear relationship between EMG activity and torque and was added to the measured torque to obtain the net extension torque (Magnusson et al., 2001). For this purpose, EMG activity was recorded from the biceps femoris muscle during knee extension and knee flexion maximal isometric contractions, and filtered offline using a second order butterfly filter with cut off frequencies of 10 and 300 Hz. Standard procedures for skin preparation were followed (Hermens et al., 1999) and surface electrodes (Ag/AgCL; 120 dB, Input impedance: 1200 GOhm; 10 mm diameter, 22 mm spacing, Biovision, Wehrheim, Germany) were placed over the biceps femoris muscle. The EMG amplitude was calculated offline as the root mean square of the signal over a 0.5 s period around the peak torque. Tendon force was calculated by dividing the net extension torque by the tendon moment arm length. Moment arm length was calculated individually from femur length (Visser et al., 1990). Tendon force-elongation relationships obtained for each trial were plotted and averaged across trials for each subject. To standardize pre- and post-test analyses, tendon stiffness and Young's modulus were calculated at the same force level, corresponding to the individual lowest peak force produced during ramped contractions. Force-elongation relationships were fitted with a second-order polynomial function and tendon stiffness (k) and Young`s modulus (E) were determined over the highest 10% force interval using Eq. (1) and Eq. (2).

$$k = \frac{\partial F}{\partial l} \qquad \text{Eq. 1}$$

$$E = k \times \frac{l_0}{CSA} \qquad \text{Eq. 2}$$

Where ∂F is the change in force and ∂l describes the tendon deformation over the force interval. CSA is the mean tendon cross sectional area and l_0 the resting tendon length. Tendon strain was calculated as the maximal tendon elongation in relation to its resting length. All relations retained for the analysis presented a coefficient of determination R^2 superior to 0.97.

Knee extension torque-angle relationship

Maximal isometric knee-extension torque was measured at five knee joint angles after tendon measurements to obtain the torque-angle relationship. Subjects had to perform two maximal isometric contractions at 50°, 60°, 70°, 80° and 90° of knee joint angle. The resting period between attempts at a given angle was 30 seconds, and it was 120 seconds between sets at different joint angles. The highest torque value at each angle was retained for further analyses. The testing order of knee angles was randomly selected but identical at pre- and post-test for each subject.

Reliability

Measurement reliability was assessed using the data from pre- and post-intervention of the iCON group by calculating the typical error of measurement (Hopkins, 2000) and the coefficient of variation (CV).

Statistics

Normality of the data distribution was checked using the Kolmogorov-Smirnov test. Baseline differences were tested for each variable with a one-way ANOVA. In case of significant between-group effect, a Bonferroni corrected post-hoc test was performed. The effects of the intervention on each variable were tested with a two-way ANOVA for repeated measures [3 (groups) x 2 (times)]. In the case of maximum isometric torque during knee extension, two-way ANOVAs were performed at each angle separately. When significant interaction effects were found, a one-way ANOVA comparing the absolute pre-post differences between each group with Bonferroni adjusted P values was performed to determine which group(s) differed over time. The level of significance was set at $P < 0.05$. Data are presented as means and standard deviations (SD).

Results

Fifty-one out of the original 55 subjects completed the study. Two subjects from the WBV, one from the iCON and one from the aCON group dropped out in the first week due to incompatibility between their personal schedule and the training/testing requirements. All the remaining subjects of the WBV and aCON groups performed the scheduled 24 training sessions over eight weeks, without any report of adverse side effects. Following the screening of ultrasound recordings, five additional subjects (2 WBV; 1 aCON; 2 iCON) had to be excluded because we could not analyze the ultrasound scans of their tendon elongation (tracked features did not remain in the field of view during contraction). The recruitment and retention of the subjects is summarized in **Figure 2**. The composition and characteristics of the groups with the remaining subjects is described in **Table 1**.

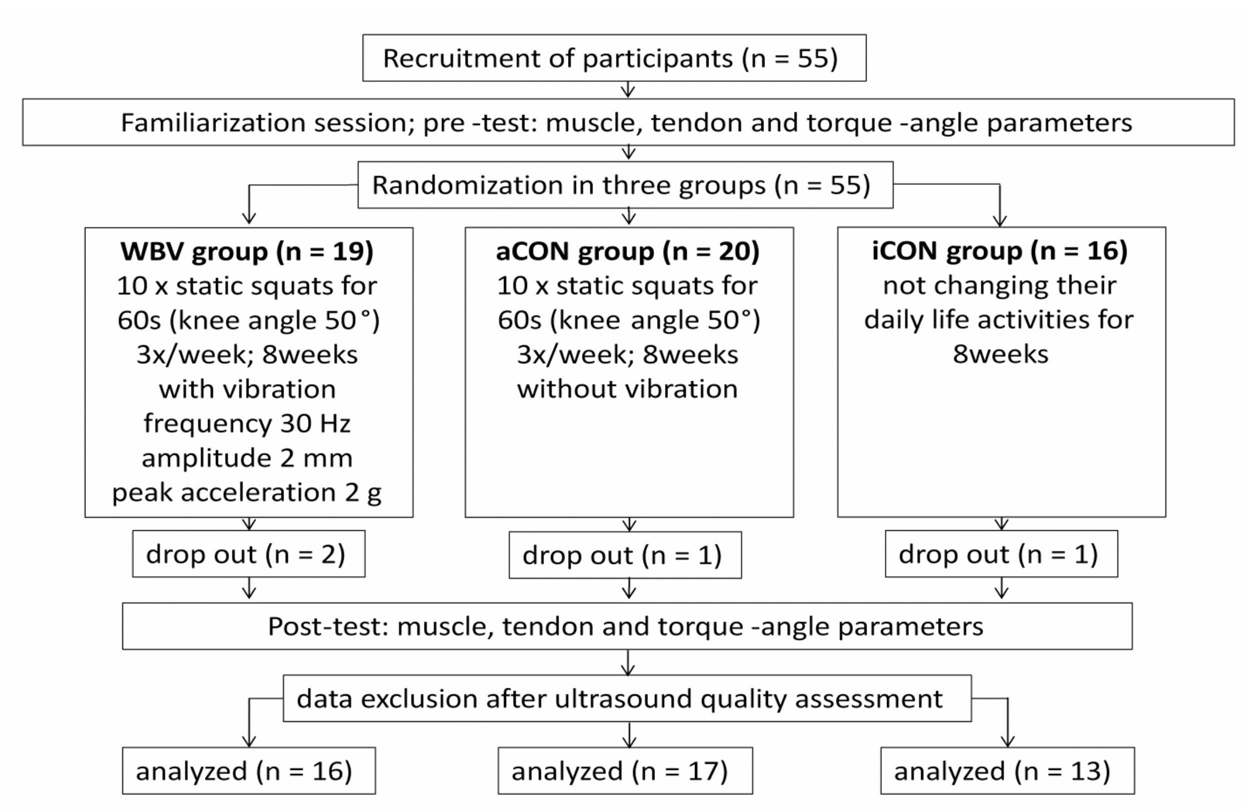

Figure 2: Flow chart illustrating participant recruitment and retention.

Table 1: Baseline characteristics.

	WBV			aCON			iCON		
Participants	N	=	16	n	=	17	n	=	13
Men	N	=	5	n	=	4	n	=	6
Women	N	=	11	n	=	13	n	=	7
Age (years)	35	±	10	33	±	11	29	±	5
Body height (cm)	171	±	8	171	±	8	177	±	11
Body weight (kg)	72	±	14	69	±	9	75	±	13
Muscle parameters									
RF CSA (mm²)	232	±	90	232	±	123	277	±	147
VL Tm (mm)	20	±	5	20	±	4	22	±	3
VL Lf (mm)	75	±	20	78	±	13	83	±	15
VL θ (°)	19	±	4	17	±	3	18	±	3
Tendon parameters									
CSA proximal (mm²)	104	±	20	101	±	21	98	±	10
CSA medial (mm²)	91	±	18	84	±	13	86	±	12
CSA distal (mm²)	104	±	22	95	±	14	100	±	18
Mean CSA (mm²)	100	±	17	93	±	15	95	±	11
Stiffness (N/mm)	1935	±	521	2157	±	449	1830	±	493
Young`s modulus (Gpa)	0.9	±	0.2	1.02	±	0.18	0.88	±	0.28
Strain (%)	8.6	±	3.6	8.8	±	2.6	9.5	±	1.6
Maximal torque									
90° (Nm)	153	±	44	154	±	42	191	±	62
80° (Nm)	175	±	51	172	±	44*	219	±	70*
70° (Nm)	193	±	58	186	±	47*	238	±	77*
60° (Nm)	192	±	61	177	±	29*	226	±	62*
50° (Nm)	174	±	58	162	±	28	200	±	44

Values are presented as mean ± SD. WBV = whole body vibration group; RF = m. rectus femoris; aCON = active control group; iCON = inactive control group; CSA = cross sectional area; VL = m. vastus lateralis; Tm = muscle thickness; Lf = fascicle length; θ = pennation angle; * = significant differences between groups ($P < 0.05$).

With the exception of knee extension torque, baseline measurements did not differ significantly between groups (all $P > 0.05$). There were significant group differences for 90°, 80°, 70° and 60° isometric knee extension torque (all $P < 0.05$). Bonferroni corrected post-hoc analyses indicated that the maximum torque of iCON and aCON subjects differed significantly ($P < 0.05$) at 80°, 70° and 60° of joint angle with higher values in the iCON group (80°: 219 ± 70Nm vs 172 ± 44Nm; 70°: 238 ± 77Nm vs 186 ± 47Nm; 60°: 226 ± 62Nm vs 177 ± 29Nm). Similarly, RF CSA values of the iCON group (277 ± 147mm²) were higher compared to the WBV

(232 ± 90mm²) and aCON group (232 ± 123mm²) but these differences did not reach significance. The reliability of these measurements is presented in **Table 2**.

Table 2: Reliability of measurements.

	TEM	CV (%)
Muscle parameters		
RF CSA (mm²)	32	7.5
VL Tm (mm)	2.1	7.3
VL Lf (mm)	10.1	7.2
VL θ (°)	1.2	6.3
Tendon parameters		
CSA proximal (mm²)	1	1.3
CSA medial (mm²)	1	1.4
CSA distal (mm²)	2	1.2
Mean CSA (mm²)	6	0.6
Stiffness (N/mm)	142	5.5
Young`s modulus (Gpa)	0.1	7.1
Strain (%)	1.3	10.1
Maximal torque		
90° (Nm)	8.8	3.5
80° (Nm)	12.9	3.8
70° (Nm)	10.9	3.8
60° (Nm)	15.0	4.4
50° (Nm)	7.6	3.3

Typical error of measurement (TEM) and coefficient of variation (CV) obtained from pre- and post-test values of the inactive control group; RF = m. rectus femoris; VL = m. vastus lateralis; CSA = cross sectional area; Tm = muscle thickness; Lf = fascicle length; θ = pennation angle.

Muscle architecture

There was no significant group by time interaction effects for RF CSA, VL Tm, VL ϴ or VL Lf (all $P > 0.05$). These data are summarized in **Table 3**.

Insert Table 3. Muscle morphological parameters at pre- and post-test. [see last pages]

Tendon morphology and mechanical properties

There were no significant group by time interaction effects for patellar tendon stiffness, Young`s modulus or strain ($P > 0.05$ in all cases, **Table 4**).

Insert Table 4. Patellar tendon mechanical properties. [see last pages]

However, there were significant group by time interactions for the proximal (CSAp: $P = 0.001$; $F_{(2,43)} = 8.50$), distal (CSAd: $P = 0.02$; $F_{(2,43)} = 4.26$) and mean ($P < 0.001$; $F_{(2,43)} = 10.08$) tendon CSA. Post-hoc analyses further indicated that in the WBV group, changes in proximal (CSAp: +6.3 ± 6.9%) and mean (+3.8 ± 3.9%) CSA were significantly different than in the aCON (CSAp: -0.9 ± 6.6%, $P = 0.001$; mean CSA: -0.4 ± 2.6%, $P < 0.001$) and iCON (CSAp: +0.8 ± 3.5%, P = 0.007; mean CSA: -0.1 ± 3.1%; $P = 0.004$) groups. The changes in distal CSA (CSAd: +5.4 ± 6.5%) seen in the WBV group only differed significantly from that of the iCON group (+0.1 ± 2.3%, $P = 0.025$). No significant differences were observed between the aCON and iCON groups (CSAp: $P = 1.000$; CSAd: $P = 1.000$; mean CSA: $P = 1.000$). The analysis of the changes at the mid-portion of the tendon did not yield any interaction effect ($P = 0.441$; $F_{(2,43)} = 0.84$, **Figure 3**).

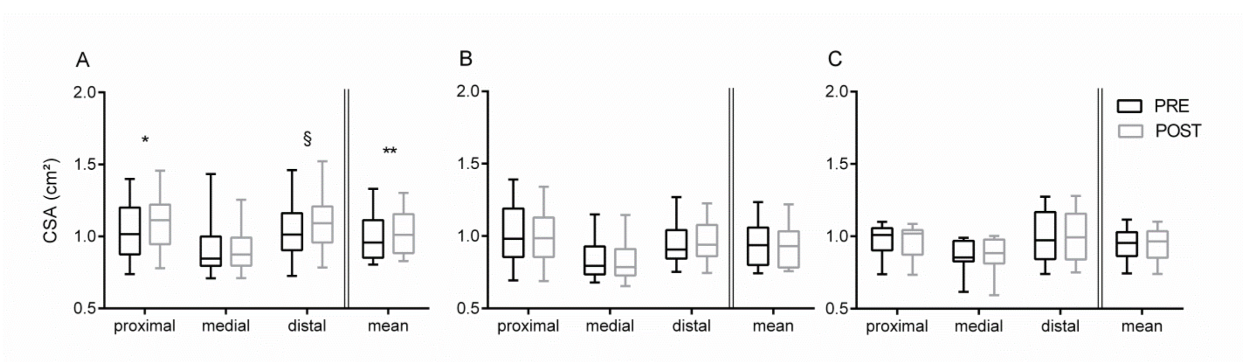

Figure 3: Changes in patellar tendon CSA.

Values are presented as mean ± SD for the whole body vibration (A), the active control (B) and the inactive control (C) groups. * = significantly different changes between pre- and post-test, when compared to the two other groups ($P < 0.05$); ** = significantly different changes between pre- and post-test, when compared to the two other groups ($P < 0.01$); § = significantly different changes between pre- and post-test, when compared to the inactive control group ($P < 0.05$).

Knee extension torque-angle relation

A significant group by time interaction ($P = 0.028$; $F_{(2,43)} = 3.91$) was found for maximum torque at 90° of knee angle. Post-hoc analyses indicated that, at this angle, changes in peak torque of the aCON group (+6.7 ± 6.4%) differed significantly from that of the iCON group (- 0.7 ± 5.6%, $P = 0.034$) but not from that of the WBV group (+6.0 ± 8.2%, $P = 1.000$). Differences between the WBV and iCON group were not significant ($P = 0.273$). No significant interaction effect was found regarding peak torque at any other knee angle (50°: $P = 0.243$; $F_{(2,43)} = 1.46$; 60° $P = 0.553$; $F_{(2,43)} = 0.60$; 70° $P = 0.641$; $F_{(2,43)} = 0.45$; 80° $P = 0.420$; $F_{(2,43)} = 0.89$, **Figure 4**). On average, the optimal knee joint angle for torque production was 67 ± 7° in all groups at baseline and 69 ± 8° after the intervention.

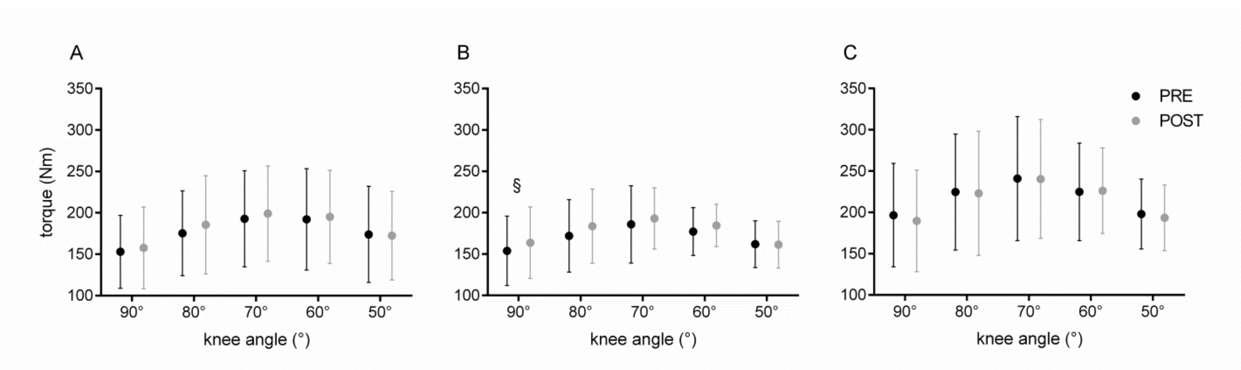

Figure 4: Torque-angle relationships.

Values are presented as mean ± SD for the whole body vibration (A), the active control (B) and the inactive control (C) groups. § = significantly different changes between pre- and post-test, when compared to the inactive control group ($P < 0.05$).

Discussion

Contrary to our hypothesis, the present protocol of WBV training did not appreciably alter knee extension torque production or the musculotendinous parameters underpinning this function. The occurrence of tendon hypertrophy, without any substantial change in mechanical properties or in muscle function, nonetheless constitutes a novel finding.

Tendon morphology and mechanical properties

It should be noted that reliability studies have highlighted certain limitations regarding ultrasound-based measurements of tendon morphology (Brushoj et al., 2006; Ekizos et al., 2013). Whilst this technique presented a satisfying inter-day reliability in our hands (CV <2%), these limitations do exist, notably due to the difficulty to obtain a sufficient contrast between tendinous and surrounding tissue. Precautions were taken to limit subjective bias, with a single, blinded, investigator performing ultrasound analysis. Hence, this cautious approach, the robust design of the present study, and the relatively larger magnitude of the changes (3.8-6.3%) compared to CVs substantiate the validity of the observed increase in tendon CSA. Indications of tendon hypertrophy have previously been observed in cross-sectional studies comparing runners (Rosager et al., 2002) or resistance-trained athletes (Seynnes et al., 2009) to controls. Similar observations were also made on the patellar tendon of individuals with asymmetric limb loading (fencers and badminton players; Couppé et al., 2008). Collectively, these studies support the hypothesis of tendon growth as a possible adaptive response to increased loading after years of exposure to an important mechanical stimulus. However, the present increase in proximal (+6.3%) and mean CSA (+ 3.8%) of the patellar tendon is remarkably similar in magnitude to observations following high-intensity resistance training (Kongsgaard et al., 2007; Seynnes et al., 2009). Although similar adaptations have also been obtained with resistive exercise of moderate-intensity (Kongsgaard et al., 2007), the fact that tendon CSA did not change significantly in the active control group indicates that the hypertrophy measured in WBV subjects cannot be ascribed to the squatting posture alone. The anabolic effect of vibration on connective tissue has been observed in a few cases, previously. An increased bone density was found after WBV training in sheep (Rubin et al., 2001) and in humans with low bone mineral density (Verschueren et al., 2004; Gilsanz et al., 2006). In addition, an anabolic response has also been observed in tendinous tissue *in vitro* (Adekanmbi et al., 2013) and in animal studies (Sandhu et al., 2011; Keller et al., 2013), supporting the present findings. The mechanisms mediating tendon growth with WBV cannot be inferred from our measurements. Processes of mechano-transduction (Kjaer, 2004) and/or microdamage-repair (Wang & Ker, 1995) cycles typically associated with a net collagen synthesis may be potentiated with high frequency, low-magnitude loading. Despite the increase in tendon CSA, the 8.1% increase in tendon stiffness of WBV subjects did not reach significance and is considerably lower compared to resistance training studies (15-84%; Wiesinger et al., 2015). By contrast, the increase in CSA measured in rats on the vibrated flexor carpi ulnaris tendons was accompanied by changes in stiffness (Sandhu et al., 2011). The present lack of significant increase in tendon stiffness could be linked to the methodological limitations of human tendon testing *in vivo*. Our reliability tests indicate that stiffness measurements can be obtained with a CV of 5.5%, which may have been too high to detect an 8% change in this variable, given our sample size. Future studies should re-examine the effect of WBV training on tendon mechanical properties with a larger sample population. Alternatively, an increase in the CSA of tendon without any change in stiffness could have been mediated by a decrease of its tensile modulus, underpinned by an accumulation of non-collagenous molecules and/or water resulting from WBV training. Consistently with reports of unchanged tendon material properties in animals subjected to WBV (Sandhu et al., 2011; Keller et al., 2013), our results do not support this hypothesis. However, additional evidence obtained via more direct mesurements of tendon composition and material properties is required to confirm the present findings and to inform about their physiological significance.

Muscle architecture

Surprisingly, the vibration training intervention did not significantly affect muscle architecture. Animal data from immobilization studies (Williams & Goldspink, 1973) or training studies in humans (McMahon et al., 2014) suggests that the number of serial sarcomeres is adjusted in function of dominant functional lengths. Since the VL muscle fascicles are arguably longer in the squatting joint configuration than during standing or walking, we expected fascicle length to adjust to these new functional requirements and vibration to potentiate this mechanical stimulus. In support of this hypothesis, muscular hypertrophy has previously been reported in older populations (Bogaerts et al., 2007) subjected to WBV training. The discrepancy between these reports and the present results probably lies in the strength of the training stimulus relative to the physical status of the subjects. The loading (e.g. time under tension, load magnitude or volume) and/or vibration parameters used in our protocol may have been insufficient to induce an appreciable anabolic response in young and healthy subjects. Furthermore, the fact that such changes were observed in the WBV group as well as in the sham vibration group or in studies without any sham vibration group is noteworthy, suggesting that WBV training may induce muscle hypertrophy via the sole isometric contractions required by the subjects' posture, not as a result of WBV training.

Torque-angle relationship

The lack of changes in muscle architecture or in tendon mechanical properties inevitably mirrored an unchanged torque-angle relationship. By contrast, Savelberg et al. (2007) observed a shift in peak torque angle towards a shorter muscle length after four weeks of WBV training in a near upright position. We predicted that a training posture requiring a relatively longer MTU would result in a shift of optimal angle in the opposite direction, towards longer muscle length, but the peak torque remained at 70° of knee flexion. A possible explanation for the unaltered angle of peak torque may lie in the similar MTU lengths during training and testing, despite different joint configurations. In line with this hypothesis, the normalized MTU length (with respect to limb length; Hawkins & Hull 1990) was calculated a posteriori, indicating that the quadriceps of the subjects was working at similar MTU length during training (115.4%) and during testing at optimal angle of torque production (115.2%).

A higher torque value was observed post-intervention at a knee angle of 90° in the WBV (6%) and the aCON (6.7%) groups. Yet this increase was only significant for the aCON group when compared to the iCON subjects (- 0.7%). The lack of change in knee extension torque contrasts with previous findings (Delecluse et al., 2003; Osawa & Oguma, 2013) showing an increase in this variable after WBV training. This discrepancy cannot be explained with the present findings but differences in training protocols such as those mentioned above are possible explanations. Whole-body vibration may have a limited influence on maximum torque production under the present training parameters, as suggested by other studies using similar protocols (De Ruiter et al., 2003; Osawa et al., 2011).

Perspective

WBV exposure without external loading does not seem to alter the torque-angle curve or the musculotendinous parameters. However, the increase in patellar tendon CSA measured in the present study suggests that this type of intervention triggers an anabolic response in tendinous tissue. An increased CSA could reduce the stress imposed by strenuous activities and increase the tendon safety factor (maximal stress imposed during daily activities/ failure stress, Ker et al., 1988). In addition, the suitability of WBV as a means to prevent injuries (e.g. in sedentary populations) or in the context of conservative approaches to treat certain tendon disorders may be investigated. Finally, future studies may address the possibility to optimize WBV training by altering vibration parameters and progressively increase the training stimulus.

Conflict of interest statement

The authors disclose that there is no potential conflict of interest, including no financial and personal relationships with other people or organizations, patent applications or consultancies that could inappropriately influence this work.

References

Adekanmbi I, Baboldashti NZ, Yapp C, Franklin S, Thompson MS. A novel in vitro loading system for high frequency loading of cultured tendon fascicles. Med Eng Phys 2013: 35: 205-210.

Blazevich AJ, Cannavan D, Coleman DR, Horne S. Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. J Appl Physiol 2007: 103: 1565-1575.

Bogaerts A, Delecluse C, Claessens AL, Cloudyzer W, Boonen S, Verschueren SP. Impact of whole-body vibration training versus fitness training on muscle strength and muscle mass in older men: a 1- year randomized controlled trial. J Gerontol 2007: 62A: 630-635.

Bojsen-Møller J, Kalliokoski KK, Seppänen M, Kjaer M, Magnusson SP. Low-intensity tensile loading increases intratendinous glucose uptake in the Achilles tendon. J Appl Physiol 2006: 101: 196-201.

Brushoj C, Henriksen BM, Albrecht-Beste E, Hölmich P, Larsen K, Bachmann Nielsen M. Reproducibility of ultrasound and resonance imaging measurements of tendon size. Acta Radiol 2006: 47: 954-959.

Colson SS, Pensini M, Espiosa J, Garrandes F, Legros P. Whole-body vibration training effects on the physical performance of basketball players. J Strength Cond Res 2010: 24: 999-1006.

Couppé C, Kongsgaard M, Aagaard P, Hansen P, Bojsen-Moller J, Kjaer M, Magnusson SP. Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. J Appl Physiol 2008: 105: 805-810.

De Ruiter CJ, Van Raak SM, Schilperoort JV, Hollander AP, de Haan A. The effects of 11 weeks whole body vibration training on jump height, contractile properties and activation of human knee extensors. Eur J Appl Physiol 2003: 90: 595-600.

Delecluse C, Roelants M, Verschueren S. Strength increase after whole-body vibration compared with resistance training. Med Sci Sports Exerc 2003: 35: 1033-1041.

Ekizos A, Papatzika F, Charcharis G, Bohm S, Mersmann F, Arampatzis A. Ultrasound does not provide reliable results for the measurement of the patellar tendon cross sectional area. J Electromyogr Kinesiol 2013: 23: 1278-1282.

Gilsanz V, Wren TA, Sanchez M, Dorey F, Judex S, Rubin C. Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. J Bone Miner Res 2006: 21: 1464-1474.

Hawkins D, Hull ML. A method for determining lower extremety muscle-tendon lengths during flexion/extension mevements. J Biomech 1990: 23: 487,494.

Hermens J, Freriks B, Merletti R, Stegeman DF, Blok J, Rau G, Disselhorts-Klug C, Hägg G. European Recommendations for Surface Electromyography. Enschede, Netherlands: Roessingh Research and Development 1999.

Hopkins WG. Measures of reliability in sports medicine and science. Sports Med 2000: 30: 1-15.

Keller BV, Davis ML, Thompson WR, Dahners LE, Weinhold PS. Varying whole body vibration amplitude differentially affects tendon and ligament structural and material properties. J Biomech 2013: 46: 1496-1500.

Kemertzis MA, Lythgo ND, Morgan DL, Galea MP. Ankle flexors produce peak torque at longer muscle lengths after whole-body vibration. Med Sci Sports Exerc 2008: 40: 1977-1983.

Ker RF, Alexander RMcN, Bennet MB. Why are mammalian tendons so thick? J Zool 1988: 216: 309-324.

Kjaer M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev 2004: 84: 649-698.

Kongsgaard M, Reitelseder S, Pedersen TG, Holm L, Aagaard P, Kjaer M, Magnusson SP. Region specific patellar tendon hypertrophy in humans following resistance training. Acta Physiol (Oxf) 2007: 191: 111-121.

Kösters A, Wiesinger HP, Bojsen-Moller J, Müller E, Seynnes OR. Influence of loading rate on patellar tendon mechanical properties in vivo. Clin Biomech (Bristol, Avon) 2014: 29: 323-329.

Kubo K, Kanehisa H, Fukunaga T. Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures in vivo. J Physiol 2002: 538: 219-226.

Lieber RL, Brown CG, Trestik CL. Model of muscle-tendon interaction during frog semitendinosis fixed-end contractions. J Biomechanics 1992: 25: 421-428.

Magnusson SP, Aagaard P, Dyhre-Poulsen P, Kjaer M. Load-dicplacement properties of the human triceps suraer aponeurosis in vivo. J Physiol 2001:531: 277-288.

McMahon GE, Morse CI, Burden A, Winwood K, L. OG. Muscular adaptations and insulin-like growth factor-1 responses to resistance training are stretch-mediated. J Strength Cond Res 2014: 28: 245-255.

Muraoka T, Kawakami Y, Tachi M, Fukunaga T. Muscle fiber and tendon length changes in the human vastus lateralis during slow pedaling. J Appl Physiol 2001: 91: 2035-2040.

Osawa Y, Oguma Y. Effects of resistance training with whole-body vibration on muscle fitness in untrained adults. Scand J Med Sci Sports 2013: 23: 84-95.

Osawa Y, Oguma Y, Onishi S. Effects of whole-body vibration training on bone-free lean body mass and muscle strength in young adults. J Sports Sci Med 2011: 10: 97-104.

Pellegrini MJ, Lythgo ND, Morgan DL, Galea MP. Voluntary activation of the ankle plantar flexors following whole-body vibration. Eur J Appl Physiol 2010: 108: 927-934.

Reeves ND, Maganaris CN, Longo S, Narici MV. Differential adaptations to eccentric versus conventional resistance training in older humans. Exp Physiol 2009: 94: 825-833.

Roelants M, Verschueren SM, Delecluse C, Levin O, Stijnen V. Whole-body-vibration-induced increase in leg muscle activity during different squat exercises. J Strength Cond Res 2006: 20: 124-129.

Rosager S, Aagaard P, Dyhre-Poulsen P, Neergaard K, Kjaer M, Magnusson SP. Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. Scand J Med Sci Sports 2002: 12: 90-98.

Rubin C, Turner AS, Bain S, Mallinckrodt C, McLeod K. Anabolism: Low mechanical signals strengthen long bones. Nature 2001: 412: 603-604.

Sandhu E, Miles JD, Dahners LE, Keller BV, Weinhold PS. Whole body vibration increases area and stiffness of the flexor carpi ulnaris tendon in the rat. J Biomech 2011: 44: 1189-1191.

Savelberg HC, Keizer HA, Meijer K. Whole-body vibration induced adaptation in knee extensors; consequences of initial strength, vibration frequency, and joint angle. J Strength Cond Res 2007: 21: 589-593.

Seynnes OR, Erskine RM, Maganaris CN, Longo S, Simoneau EM, Grosset JF, Narici MV. Resistance training patella tendon muscle hypertrophy. J Appl Physiol 2009: 107: 523-530.

Torvinen S, Kannus P, Sievanen H, Jarvinen TA, Pasanen M, Kontulainen S, Jarvinen TL, Jarvinen M, Oja P, Vuori I. Effect of four-month vertical whole body vibration on performance and balance. Med Sci Sports Exerc 2002: 34: 1523-1528.

Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. J Bone Miner Res 2004: 19: 352-359.

Visser JJ, Hoogkamer JE, Bobbert MF, Huijing PA. Length and moment arm of human leg muscles as a function of knee and hip-joint angles. Eur J Appl Physiol 1990: 61: 453-460.

Wang XT, Ker RF. Creep rupture of wallaby tail tendons. J Exp Biol 1995: 198: 831-845.

Wiesinger H, Kösters A, Müller E, Seynnes OR. Effects of increades loading on in vivo tendon properies: a systematic review. Med Sci Sports Exerc 2015: [Epub ahead of print] PMID: 25563908.

Williams PE, Goldspink G. Changes in sarcomere length and physiological properties in immobilized muscle. J Anat 1973: 127: 459-468.

Table 3: Muscle morphological parameters at pre- and post-test.

	WBV		aCON		iCON			
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	$F_{(2,43)}$-value	*P*-value
RF CSA (mm²)	232 ± 90	236 ± 94	232 ± 123	221 ± 109	277 ± 147	267 ± 139	1.07	0.352
VL Tm (mm)	20 ± 5	20 ± 5	20 ± 4	20 ± 3	22 ± 3	21 ± 4	1.88	0.165
VL Lf (mm)	75 ± 20	81 ± 20	78 ± 13	85 ± 21	83 ± 15	81 ± 12	2.10	0.134
VL θ (°)	19 ± 4	17 ± 3	17 ± 3	17 ± 3	18 ± 3	18 ± 4	2.12	0.132

Values are presented as mean ± SD. WBV = whole body vibration group; aCON = active control group; iCON = inactive control group; RF = m. rectus femoris; VL = m. vastus lateralis; CSA = cross sectional area; Tm = muscle thickness; Lf = fascicle length; θ = pennation angle.

Table 4: Patellar tendon mechanical properties.

	WBV		aCON		iCON			
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	$F_{(2,43)}$-value	*P*-value
Stiffness (N/mm)	1935 ± 521	2048 ± 487	2157 ± 449	2137 ± 391	1830 ± 493	1859 ± 576	0.74	0.482
Young`s Modulus (Gpa)	0.86 ± 0.24	0.87 ± 0.24	1.02 ± 0.18	1.03 ± 0.25	0.88 ± 0.28	0.91 ± 0.34	0.03	0.968
Strain (%)	8.6 ± 3. 6	7.8 ± 1.7	8.8 ± 2.6	8.7 ± 2.4	9.5 ± 1.6	9.6 ± 1.8	0.69	0.506

Values are presented as mean ± SD. WBV = whole body vibration group; aCON = active control group; iCON = inactive control group.