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**Eight weeks of local vibration training do not increase tibialis anterior muscle stiffness evaluated by supersonic shear imaging**

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Running title: Muscle stiffness after local vibration training

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## **Abstract**

*Background:* Local vibration (LV) training is efficient to improve muscle strength due to adaptations within the central nervous system. However, little is known about adaptations at the muscular level after this form of training. The aim of this study was to assess the effect of LV training on muscle elastic properties using supersonic shear imaging technique.

*Methods:* Twenty-eight subjects were allocated to a training (VIB, n = 14) or control (CON, n = 22) group. The VIB group performed twenty-four 1-h sessions (3 sessions/wk) of 100-Hz vibration applied to the tibialis anterior. Maximal force (MVC) as well as active and passive muscle stiffness (i.e. using elastography) were assessed before and after the LV training.

*Results:* MVC was increased by  $9.4 \pm 9.7\%$  in VIB ( $p < 0.001$ ) while no changes were reported in CON ( $p = 0.52$ ). No changes were reported in passive and active muscle stiffness for both groups ( $p > 0.05$ ).

*Conclusion:* Our results suggest that adaptations in elastic muscle properties do not explain the increased muscle strength reported after LV training.

**KEYWORDS:** local vibration training; tibialis anterior; muscle strength; muscle stiffness; elastography; supersonic shear imaging

## **1. Introduction**

Local vibration (LV) applied to a muscle or its tendon is well known to strongly activate muscle spindles primary afferents [1] and may in turn induce a “tonic vibration reflex” [2]. After prolonged exposure to LV (i.e. 20-60 min), a decrease in maximal-force generating capacity has been commonly reported (for a review see Souron et al. [3]). Then, when LV sessions are repeated over days or weeks, it has been reported to trigger long-term adaptations as indicated by increased strength [3]. While such an increase in force capacities was mainly discussed through adaptations within the central nervous system [3], adaptations at the muscular level have been less documented. Direct [4] and indirect data [5] suggest that hypertrophy does not occur after LV training. However, changes in musculo-tendinous stiffness (i.e. within the series elastic component) may also contribute to changes in muscular performance, a stiffer musculo-tendinous unit being associated to greater force production capacities [6]. Using a quick-release test, we previously reported a decrease in triceps surae musculo-tendinous stiffness after 14 days of LV training [7]. This result was however somewhat paradoxical, and interpretation was further limited by the used experimental technique that can only document about the global mechanical properties of the musculo-tendinous complex with no distinction in the contribution of the various structures involved, i.e. muscles, tendon, joint [7].

Muscle stiffness may be specifically investigated in vivo through elastography techniques by measuring shear wave propagation velocity [8]. Using supersonic shear imaging (SSI), tissue stiffness (i.e., Shear modulus, in kPa) is calculated from the squared velocity of shear waves remotely induced by focused ultrasound [9]. It may for instance inform about passive muscle stiffness in a variety of resting muscles [10]. Because stiffness at the muscle fiber level is directly related to the number of attached cross bridges during a contraction [11], we previously proposed to calculate an index of muscle active stiffness as the slope of the relationship between muscle shear modulus and force [12, 13]. Such index was proposed to be potentially related to

the active part of the series elastic component. Then, it remains to be determined whether LV training may induce changes in active muscle stiffness as investigated with SSI. Therefore, the aim of the present study was to investigate the effect of LV training on active and passive muscle stiffness of the tibialis anterior, a functionally important muscle [14].

## **2. Material and Methods**

### ***2.1. Subjects***

Twenty-eight, healthy subjects (14 men and 14 women; age:  $20 \pm 1$  year, height:  $171 \pm 10$  cm, body mass:  $64 \pm 10$  kg) participated in this study. This study was carried out in accordance with the recommendations of the local ethics committee (CPP Sud Est I NCT02668224) with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. All participant were free of lower limb injury during the previous 3 months.

### ***2.2. Elastography***

Muscle shear modulus was measured using an AixPplorer ultrasonic scanner (version 6.1.1, Supersonic Imagine, Aix en Provence, France), coupled with a linear transducer array (4–15 MHz, SuperLinear 15–4, Vermon, Tours, France). The scanner was used in the musculo-skeletal preset of the SSI mode, as previously described [9, 15]. Briefly, the velocity ( $V_s$ ) of a shear wave, induced by a remote radiation force, is measured along the principal axis of the probe. The shear modulus ( $\mu$ ) was calculated using  $V_s$  as follows [16]:

$$\mu = \rho \cdot V_s^2$$

with  $\rho$  the muscle mass density ( $1000 \text{ kg/m}^3$ ).

The ultrasound probe was placed on the tibialis anterior (TA) belly, centered at 40% of the length from the popliteal crease to the center of the lateral malleolus [17]. The probe was carefully aligned with the shortening direction of the muscle, and perpendicular to the skin. The

location of the probe was marked on the skin to allow the same placement across trials. Maps of the shear modulus (Fig 1) were obtained at 1Hz with a spatial resolution of  $1 \times 1$  mm.

### ***2.3. Torque recordings***

Dorsiflexion torque was measured during voluntary contractions by a calibrated instrumented pedal (CS1060 300 Nm, FGP Sensors, Les Clayes Sous Bois, France). Subjects were seated upright in a custom-built chair with pelvis, knee and ankle angulations of 90, 120 and 90°, respectively. Signals were analogue-to-digitally converted at a sampling rate of 2000 Hz by PowerLab system (16/30—ML880/P, ADInstruments, Bella Vista, Australia) and analyzed offline using Labchart 7 software (ADInstruments).

### ***2.4. Design of the study***

Subjects were randomly assigned to either the control (CON;  $n = 14$ ) or the vibration (VIB;  $n = 14$ ) group. All the subjects performed the experimental procedures (see below) during two testing sessions. After a pretraining test session (PRE), the subjects of the VIB group followed an 8-week LV training program, for a total of 24 LV sessions. Subjects were retested at the end of the training (POST). The vibrating device (VB 115; Techno Concept, Mane, France) was applied locally and strapped directly on the largest part of the resting TA muscle of the right leg. The subject was seated on a chair during the entire vibration session, with a knee angle of 90°. The LV training program consisted of three 1-h vibration sessions per week (frequency: 100 Hz; amplitude: 1 mm) with at least 1 day of rest between consecutive sessions. No specific recommendations were given to the subjects of the CON group, who were asked not to change their usual activities for the duration of the study.

### ***2.5. Experimental procedures***

Subjects first performed three 3-s maximal isometric voluntary dorsiflexion (1-min rest between contractions). Maximal torque was considered as the highest peak torque from the three trials. Then, passive stiffness was assessed (i.e. 10 maps recorded) by placing the

ultrasound probe on the relaxed TA while the subject was seated on a chair. Shear modulus was calculated in the selected circular area placed on the TA (approximately 1 cm in diameter) using the Aixplorer scanner software (Q-Box<sup>TM</sup>) (Figure 1). Thereafter, one set of submaximal dorsiflexion was performed to evaluate active muscle stiffness. Subjects performed three 5-s trials at each of 20,30,40,50 and 60% MVC in a random order (30-s and 60-s intervals between trials and torque level, respectively). Torque and shear modulus were synchronously recorded for each trial once the torque and map of elasticity were stable. For each subject, a linear regression analysis was then performed between TA shear modulus and dorsiflexion torque (see Fig 2 in Souron et al. [13]), and active stiffness was calculated as the slope of the curve [12, 13].

## **2.6. Statistics**

All variables were normally distributed (Kolmogorov- Smirnov normality test). For ANOVA analyses, homogeneity of variance was verified by Levene's test. Two-way repeated measures ANOVAs were performed [group (CON or VIB)  $\times$  time (PRE, POST)]. Post hoc analyses were performed using Newman-Keuls testing when the ANOVAs identified significant differences. Partial eta square ( $\eta^2$ ) was reported as an estimate of effect size, with  $\eta^2 \geq 0.07$  and  $\eta^2 \geq 0.14$  used as moderate and large effects, respectively [18]. Statistical significance was set at  $p < 0.05$ .

## **3. Results**

There was a significant group  $\times$  time interaction for MVC ( $p = 0.02$ ;  $\eta^2 = 0.34$ ). In VIB, MVC was significantly increased at POST by  $9.4 \pm 9.7\%$  when compared to PRE ( $p < 0.001$ ), while no significant differences were observed for CON ( $p = 0.52$ ). No significant group  $\times$  time interactions were found for passive ( $p = 0.86$ ;  $\eta^2 = 0.01$ ) and active stiffness ( $p = 0.65$ ;  $\eta^2 = 0.01$ ) (Table 1).

**Table 1.** Maximal voluntary contraction (MVC) and active and passive stiffness indexes recorded before (PRE) and after (POST) training period for control (CON) and vibration (VIB) groups.

	CON		VIB	
	PRE	POST	PRE	POST
MVC (Nm)	58.8 ± 18.2	59.5 ± 18.1	52.3 ± 13.9	57.3 ± 16.4***
Active stiffness (kPa.Nm <sup>-1</sup> )	4.1 ± 1.2	3.9 ± 1.0	4.7 ± 2.8	4.6 ± 2.4
Passive stiffness (kPa)	40.6 ± 13.0	40.2 ± 13.4	40.5 ± 13.4	42.9 ± 20.2

Significantly different from PRE (main interaction effect): \*\*\*p < 0.001.

#### 4. Discussion

The present study confirms that LV training is an appropriate method to improve MVC in healthy individuals [3]. We recently reported that adaptations within the central nervous system are largely involved in the increased force generation capacities [19]. The aim of this study was therefore to assess if the increased muscle strength after LV training may rely on modifications in muscle elastic properties using SSI recordings.

First, no changes in active muscle stiffness were reported in this study. If this results contrasts with one of our recent work that documented a decrease in triceps surae musculo-tendinous stiffness after 14 days of LV training [7], one should keep in mind that different structures were assessed in these two studies, i.e. global mechanical properties of the musculo-tendinous complex (e.g. muscles, tendon, joint) in the study of Lapole and Pérot [7] versus muscle properties in this work. Moreover, differences in the tested muscular group and LV training characteristics may also account for such discrepancies. When considering the effects of plyometric training, changes in active muscle stiffness have been investigated using a fast stretch during submaximal voluntary isometric contractions [20, 21]. Using the alpha method, Foure and colleagues reported a decreased active stiffness after 14 weeks of plyometric training [20] while Kubo and colleagues reported an increased active stiffness after 12 weeks of training



by directly measuring fascicle length during the stretch [21]. Then, differences in experimental procedures may also lead to different results, what may be the case with the use of shear modulus measurements in the present study. Stiffness changes with training have mainly discussed in terms of potential training-induced changes in fiber-type distribution, cross bridges of slow-twitch fibers being stiffer than for fast ones [22]. Then, the current results suggest that the work load imposed by LV training was not strong enough (likely due to the low vibration amplitude) to induce changes in muscle typology then in muscle stiffness. It should be however emphasized that the influence of fiber type on shear modulus measurement remains speculative. Further, shear modulus measurement may be influenced by other structures than cross bridges, e.g. connective tissue, fatty infiltration [12].

Second, we reported no changes in TA passive muscle stiffness after LV training, which contrasts with our previous results of a decrease in musculo-tendinous passive stiffness (i.e. investigated through passive torque-angle relationships) of the triceps surae after LV training [7]. Other studies reported a decrease in passive muscle stiffness after a 4-week static stretching program involving hamstring muscles [23] or 14-week plyometric training of gastrocnemii muscles [24]. Passive muscle stiffness has been linked to the mechanical properties of connective tissue elements in parallel with the muscle belly [25] and titin isoforms [26]. The present results suggest that LV training is unable to trigger such adaptations at the muscle level that would have led to changes in passive muscle stiffness measured by SSI.

## **5. Conclusion**

Although this study did not assess the global stiffness of the musculo-tendinous system, our results suggest for the first time that LV training is not appropriate to induce some changes in both passive and active TA muscle stiffness. These results suggest that the increase in muscle strength after LV training may be exclusively due to neural rather than muscle adaptations.

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### **Figure caption.**

**Figure 1.** Schematic representation for the position of the ultrasound probe on the tibialis anterior (TA) muscle. Typical example of shear modulus maps assessed before the training program VL. The colored region represents the shear elasticity map with the scale (in kPa) displayed on the top right of the figure (blue and red indicating soft and stiff tissues, respectively). A representative averaged shear modulus value representing the index of stiffness was obtained in the region of interest (dotted white circle).

$\geq 80$  kPa

60

40

20

0

TA

