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ABSTRACT

Purpose: Whether muscle stiffness is influenced by fatigue remains unclear. Classical methods used to assess muscle stiffness provide a global measure at the joint level. As fatigue may selectively affect specific muscles, a joint-level approach may not be sensitive enough to detect potential changes in muscle stiffness. Taking advantage of ultrasound shear wave elastography, this study aimed to determine the influence of a fatiguing protocol involving intermittent submaximal isometric contractions on muscle shear modulus (an index of stiffness).

Methods: Shear modulus was measured on either the *vastus lateralis* (VL; n=9) or the *abductor digiti minimi* (ADM; n=10) before and after 15-mins of intermittent submaximal isometric contractions at 60% of maximal voluntary contraction (MVC; 4 s ON, 4 s OFF). An index of active muscle stiffness was estimated PRE and POST-fatigue as the slope of the linear regression established between shear modulus and absolute joint force up to 60% MVC.

Results: After the fatiguing exercise, MVC was significantly decreased by 22 ± 7 % and 32 ± 15 % for knee extension and little finger abduction, respectively ($p < 0.001$). When compared to PRE-fatigue, the index of active muscle stiffness was 12 ± 15 % lower for the VL ($p < 0.031$) and 44 ± 19 % lower for the ADM ($p < 0.001$) POST-fatigue.

Conclusion: Although the present results cannot clearly determine the involved mechanisms, they demonstrate a decreased active muscle stiffness after a fatiguing task involving intermittent submaximal isometric contractions. Further studies should now determine whether this change in stiffness affects performance and risk of injury.

KEYWORDS

Fatigue; muscle stiffness; shear wave elastography; shear modulus

INTRODUCTION

Fatigue involves a progressive change within the central nervous system and/or muscle during exercise that culminates in a decrease in muscle force output (1). Whether the stiffness of the active part of the series elastic component described by the Hill's muscle model (2) is affected by fatigue remains largely unknown. Changes in muscle stiffness can theoretically affect muscle performance as stiffness plays a key role in the transmission of tension generated by the contractile component of muscles to the body segments (3).

Several experimental techniques (e.g., quick-release, dynamic perturbations) may be used to assess global stiffness at the joint level. These techniques have been used to assess the effect of fatigue on global stiffness, and conflicting results have been reported with either no effect (4, 5) or a decrease in stiffness measured at the joint level (6, 7). Interpretation of these discrepancies requires consideration of the well-known task-dependency of fatigue (8). Even though no change in global joint stiffness was reported after sustained isometric fatiguing protocols (4, 5), decreased stiffness was reported after dynamic (6) and intermittent submaximal isometric contractions (7). Also, as fatigue may selectively affect specific muscles, a global measure of stiffness at the muscle-tendon unit level (i.e., using an ergometer and without differentiating the various structures such as muscles and tendons) may not be sensitive enough to detect potential changes in stiffness at the level of individual muscles. Elastography methods provide the opportunity to non-invasively estimate mechanical properties of a localized area of soft tissue (9). Ultrasound Shear Wave Elastography (SWE) provides a reliable estimate of muscle shear modulus, an index of muscle stiffness (10). A strong linear relationship between muscle shear modulus and muscle force has been reported (11–15), which aligns with *in-vitro* experiments performed on stimulated frog muscle fibre and showing that the active stiffness at the muscle

fibre level is proportional to the number of attached cross-bridges (16). Based on this result, we previously proposed the calculation of an index of active muscle stiffness as the slope of the relationship between muscle shear modulus and joint force (11, 13). After fatigue induced by a sustained submaximal contraction performed at 40% of maximal voluntary contraction (MVC), no change in the relationship between shear modulus and force was found for the *abductor digiti minimi* muscle (17), suggesting that fatigue does not alter muscle stiffness. Yet, active muscle stiffness has never been investigated in the context of fatigue induced by intermittent contractions. (11, 13)(11, 13)(11, 13)

The aim of the present study was to determine the effect of a fatiguing protocol involving intermittent submaximal isometric contractions on active muscle stiffness measured using ultrasound SWE. As suggested from global stiffness measurements performed at the joint level (7), we hypothesized that the index of active muscle stiffness would be reduced in the presence of fatigue. We tested two different muscles during separate experiments. The *abductor digiti minimi* (ADM) is the only synergist responsible for the little finger abduction and was therefore an ideal candidate for investigating shear modulus-joint force relationships pre- and post-fatigue. For instance, potential changes in shear modulus may originate from non-uniform distribution of force across synergist muscles (17, 18). We also investigated the *vastus lateralis* (VL) as part of the functional knee extensor muscle group, which experiences more fatigue in daily life and sport activities than the ADM.

METHODS

Participants

Measurements were performed on the *vastus lateralis* in nine healthy males (24 ± 4 yr, 180 ± 7 cm, 73 ± 8 kg) and on the *abductor digiti minimi* in ten healthy males (25 ± 2 yr, 178 ± 6 cm, 73

± 5 kg). All of the participants gave their written consent to participate in this study that conformed to the standards from the latest revision of the *Declaration of Helsinki* and was approved by the local ethics committee (CPP Sud Est 1).

Experimental Design

The experimental design was identical for the VL muscle during isometric knee extension and the ADM muscle during isometric little finger abductions (Figure 1). The participants first performed a near-maximal contraction (90% of maximal perceived effort) to determine the warm-up intensity. Subsequently, participants performed a 5-min standardized warm up, consisting of repeated isometric contractions (4s ON, 4s OFF) progressively increasing from 10 to 50% of the pre-warm up maximum force. After 3 minutes of rest, subjects performed two 4-s isometric maximal voluntary contractions (MVC) interspersed by 2 min of rest to determine the maximal force. If the difference between the two MVC was superior to 5%, the participants were asked to perform another MVC. During MVC measurements, the position of the subjects was standardized, and they received verbal encouragements. Thereafter, participants performed the pre-fatigue active muscle stiffness testing twice (described below) with a 3-min recovery period, in order to assess the reliability of the measurement. The active muscle stiffness was then reevaluated 10 min after the fatigue procedure.

The fatigue procedure was similar to the experimental protocol employed by Zhang et al. (7). The participants were asked to hold 60% of MVC for 6 s and to relax for 4 s. This was done for three sets lasting 6 min, 6 min and 3 min, respectively, interspersed by 2-min recovery periods. MVC was reevaluated 30 s before the start of the second and third sets to adjust the 60% target that inevitably decreased due to fatigue. Force signals and the defined target were displayed on a screen in front of the participants. To ensure that the duty cycle was respected, an audio track

gave instructions to “contract” and “relax”. After the fatigue procedure, a 10-min rest period was allowed to ensure recovery from the acute ischemic phase of fatigue (7). Subsequently, MVC was recorded in order to assess the muscle fatigue (i.e. force capability decrease) and was followed 30 s later by the last active muscle stiffness evaluation. The 10-min recovery period further allowed active muscle stiffness measurements to be performed with greater accuracy, since taking measures immediately following the fatiguing protocol would have impeded the ability of participants to reach the desired contraction level, with a potential for muscle tremor.

Force measurements

For knee extension, force was measured by a calibrated force transducer (Meiri F2732 200 daN, Celians, Montauban, France) with an amplifier attached by a non-compliant strap to the leg just proximal to the malleoli. Participants were seated upright in a custom-built chair with both hips and knees flexed at 90°.

The apparatus used to assess the little finger abduction force was previously described in Bouillard et al. (12). In brief, the participants were seated with their right elbows flexed to 120° (180° corresponds to the full extension of the elbow). The right forearm was pronated and supported on a platform, and all fingers were extended with the palm facing down. The hand and fingers 2–4 were immobilized with Velcro straps to prevent any movement and compensation during contractions. The little finger was in contact with a rigid interface, with the proximal interphalangeal joint aligned with the force sensor (SML-50, Interface).

For both muscles, signals were analogue-to-digitially 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).

Maximal force in abduction of the little finger and knee extension was calculated over a 500-ms interval centered at maximal force of the highest MVC performed pre-fatigue and the MVC post-fatigue.

Elastography

Muscle shear modulus was measured using an Aixplorer ultrasonic scanner (version 6.1.1, Supersonic Imagine, Aix en Provence, France), coupled with a linear transducer array (4–15 MHz, field of view: 5.4 cm, SuperLinear 15–4, Vermon, Tours, France). The scanner was used in the musculo-skeletal preset of the SWE mode (options: penetration, no persistence, and no spatial smoothing). In brief, the shear wave velocity (V_s), induced by a remote radiation force, was measured along the principal axis of the probe. The shear modulus (μ) was calculated using V_s as follows (19):

$$\mu = \rho V_s^2 \quad (1)$$

where ρ is the assumed muscle density (1000 kg/m^3). For ADM measurements, the ultrasound probe was placed medially, between the carpometacarpal and the metacarpophalangeal joints of the little finger. For VL measurements, the probe was placed approximately $2/3$ distally and $3/4$ laterally considering the thigh segment. These locations allowed the probe to be placed on the region with the greatest muscle thickness and avoided hypoechoic regions related to dense connective tissues (18). The probe was continually aligned to the muscle fiber shortening direction during the contraction (20), and carefully held perpendicular to the skin with a constant pressure of the probe on the skin. These locations of the probe were marked on the skin to allow the same placement across trials. Maps of the shear modulus were obtained with a spatial resolution of $1 \times 1 \text{ mm}$. The shear modulus map was chosen to be as large as possible, depending on the muscle depth/thickness to obtain a representative averaged shear modulus value (12, 17).

A trigger input was sent by the Aixplorer scanner to the PowerLab system at each shear modulus measurement such that the force and shear modulus data could be synchronized.

Active muscle stiffness measurements

For active muscle stiffness measurement of the ADM, the participants were asked to perform an incremental isometric task consisting of a 20-s smooth linear force ramp from 0 to 60% of MVC (11, 12). This maximal force of 60% of MVC corresponds to the upper limit after which one reaches saturation of the elastography measurements for all the subjects (discussed below). Shear modulus was recorded before (i.e. three recordings to control for resting ADM stiffness) and during the ramp at a 1 sample/sec. For VL muscle, pilot experiments revealed that muscle deformations induced by the ramp contractions made it difficult to maintain the position of the probe. Measurements were thus performed during force-matched isometric contractions. The participants were asked to maintain 10, 20, 30, 40 50 and 60% of MVC for 5 s in random order (10-s interval between contractions) (13). Force and shear modulus maps were synchronously recorded for each trial once they were stable. Thereafter, participants were asked to perform an extra contraction with an identical absolute force pre and post fatigue in order to compare shear modulus values at a given force level. The force targets were set at 40% for VL and 30% for ADM of the pre-fatigued MVC. These targets were chosen to ensure absence of saturation of the SWE measurement (based on preliminary experiments).

Data processing of shear modulus measurements was performed using MATLAB scripts (Mathworks, Natick, MA). Maps of the shear modulus were exported from the software in “mp4” format, sequenced in “jpeg”. A region of interest (ROI) was defined in each shear modulus map as the largest muscular region available, avoiding aponeurosis, tendon, and bone (Figure 2). For the vast majority of the pictures, the ROI was defined as the whole elastography

map ($\sim 1 \times 1.5$ cm for ADM and $\sim 1.5 \times 1.5$ cm for VL) and was kept similar between pre- and post-exercise. A mean shear modulus value was calculated over this ROI. Due to limitations of the current version of the ultrasound scanner, shear modulus measurements saturated at 266 kPa. Once any value within the ROI reached this maximum, the data of this ROI was discarded from further analysis. As a result, shear modulus values at 60% MVC in knee extension were discarded for 7 subjects while during little finger abduction saturation was observed at $43 \pm 17\%$ of MVC.

For knee extension, mean force at each target level was averaged over the 1-s period that preceded the acquisition of the shear modulus map and then averaged to obtain a representative value. Then, a linear regression was performed between mean VL shear modulus values and knee extension absolute force (Fig 1A). For each little finger abduction task, linear regression analyses were performed between ADM shear modulus and absolute force (Fig 1B). The slopes of the linear regressions obtained for both muscles were calculated to represent the active muscle stiffness index (11, 13). The slope was considered as a stiffness index to provide a stiffness evaluation that is independent from the force level (11). The shear modulus was also calculated at 40% MVC for VL (k40) and 30% MVC for ADM (k30).

Statistical analysis

Coefficients of determination (R^2) were calculated for each linear fitting. Reliability of the stiffness indexes evaluated before the fatigue procedure was assessed by the intraclass correlation coefficient (ICC) and coefficient of variation (CV), as recommended by Hopkins (21). The normality of the error distribution was examined with Lilliefors' test. Homogeneity of variance was verified using Levene's test. With the assumption of normality and homogeneity of variance confirmed, the fatigue effect was tested with a Student t-test on repeated measure (pre

vs. post-fatigue) for MVC, shear modulus at a given force level (k40 and k30) and active muscle stiffness index of both muscles. We also compared the resting ADM shear modulus. The Cohen's *d* effect-size (22) was determined and values of $d = 0.2$, 0.5 and 0.8 should be interpreted as small, medium and large effects, respectively. Data are presented as means \pm SD. Statistical significance was set at $p < 0.05$.

RESULTS

The ICC and CV of ADM resting shear modulus was 0.915 and 4.9 ± 3.4 %, respectively. Mean R^2 of the linear regression between muscle shear modulus and force was 0.97 ± 0.02 for VL and 0.94 ± 0.03 for ADM (i.e. when pooling pre- and post-fatigue measurements). The ICC and CV of the active muscle stiffness index was 0.95 and 6.6 ± 3.8 % for VL and 0.93 and 6.1 ± 4.5 % for ADM.

After the fatiguing exercise, MVC was significantly decreased by 22 ± 7 % and 32 ± 15 % for knee extension (pre-fatigue: 595 ± 83 N; post fatigue: 464 ± 49 N ; $p < 0.001$; $d = 2.04$) and little finger abduction (pre-fatigue: 24 ± 8 N; post fatigue: 17 ± 6 N ; $p < 0.001$; $d = 1.24$), respectively. After the fatigue procedure, the shear modulus at a given absolute force value was lower for VL (-16.8 ± 19.1 %; $p = 0.036$, $d = 0.99$; Figure 3A) and ADM (-37.9 ± 16.7 %; $p < 0.001$, $d = 2.09$; Figure 3B), respectively. Furthermore, active muscle stiffness index was 12 ± 15 % lower for the VL muscle ($p < 0.031$, $d = 0.88$; Figure 4A) and 44 ± 19 % lower for the ADM muscle ($p < 0.001$, $d = 1.88$; Figure 4B). ADM resting shear modulus was not statistically different between pre- and post-fatigue (10.1 ± 2.5 vs. 9.7 ± 2.3 kPa; $p = 0.520$, $d = 0.18$).

DISCUSSION

The aim of this study was to investigate the changes in active muscle stiffness of the VL and the ADM muscles following a 15-min fatiguing task. Both the shear modulus values measured at a

given absolute force level and the stiffness index decreased after the fatiguing exercise, demonstrating that active muscle stiffness is decreased after a fatiguing task involving intermittent submaximal isometric contractions.

The index of active muscle stiffness measured with either multiple stable levels of joint force (*VL*) or a ramp contraction (*ADM*) were highly reliable ($ICC > 0.90$; $CV < 7\%$). This is in accordance with previous results obtained with multiple stable levels of joint force for *tibialis anterior* (13) and with ramps for *first dorsal interosseous*, *abductor digiti minimi* (12) and *biceps brachii* (11) muscles.

Ten minutes after the fatiguing task, fatigue was still present as evidenced by the 22% decrease in knee extensor MVC and the 32% decrease in little finger abduction MVC. This is in agreement with studies reporting reduced MVC up to 30 min after a fatiguing task involving intermittent submaximal isometric contractions (23). In the present study, fatigue was associated with a decrease in shear modulus recorded at an absolute force level and a decrease in the index of active muscle stiffness, i.e. the slope of the relationship between shear modulus and joint force. This was observed for all the tested subjects, except one who demonstrated higher *VL* shear modulus values with fatigue. The origin of this change is unknown. Several parameters may have explained the observed decrease in active muscle stiffness measured using shear wave elastography. First, intramuscular temperature is known to modify muscle mechanical properties, with an increased muscle temperature being associated with a reduced stiffness measured at rest (24). To the best of our knowledge, no study has explored the effect of muscle temperature on active muscle stiffness. Even though we did not measure muscle temperature in the present study, *ADM* resting stiffness was not significantly affected by fatigue. While the possibility that temperature may differentially affect passive and active muscle stiffness cannot be ruled out, we

suggest that the increased muscle temperature associated with prolonged contraction is unlikely to fully explain the observed decreased active muscle stiffness. Second, calculation of the index of active muscle stiffness was made with the assumption that joint force is representative of individual muscle force. However, fatigue may have induced a change in the force sharing between the individual heads of the quadriceps (18), with a possible decrease in VL's force contribution that would have contributed to the decrease in shear modulus. However, the results obtained for little finger abduction, for which change in force sharing is very unlikely (ADM has no synergist muscles), suggest that changes in load sharing are unlikely to explain the observed decrease in the index of active stiffness. Third, the prolonged fatiguing task could have induced tendon creep (25), leading to an increase in the VL pennation angle during the measurements. This would have led to a decrease in shear modulus, while an increase was found in our study. However, tendon creep is generally, though not always (26), observed in the very first contractions with no further alteration in tendon behavior thereafter (27). We suggest that in the present study this tendon creep should have occurred during the warm-up procedure and may not have influenced active muscle stiffness index measurements. Moreover, a decrease in active muscle stiffness index was found for the ADM after the fatiguing task, suggesting that these changes occur independently from changes in pennation angle due to its fusiform architecture. We also acknowledge that the SWE technique provides measurements of a localized muscle region, and we cannot exclude that our measures are not fully representative of the whole muscle, especially for VL. As an example, strength training may induce regional changes related to hypertrophy and muscle architecture in a contraction-specific manner (28). Whether such regional changes in muscle stiffness may occur, especially in the context of fatigue, warrants further investigation. Nonetheless, the similar behavior of almost all participants and muscles,

especially for the small ADM muscle, provides assurance that a true effect of fatigue on active muscle stiffness was observed in the present study. Taking these factors into consideration, we are confident that our results reflect actual decrease in intrinsic active muscle stiffness. Further studies should now determine how this altered muscle stiffness could affect muscle performance (e.g. rate of force development) and the risk of injury.

Our results are in accordance with previous findings of decreased global stiffness of the elbow extensor muscles reported by Zhang et al. (7), who used dynamic perturbations after a similar fatiguing task as in the present study. Decreased global stiffness of the elbow flexors was also reported using the quick-release method after 11 sets of 45-s rhythmic flexion movements performed at a constant power (6). Conversely, ankle joint stiffness assessed using dynamic perturbations has been reported to be unaltered by a sustained 50% MVC submaximal isometric dorsiflexion maintained up to exhaustion (4), as was head-neck segment global stiffness using the quick release method after five 30-s 50% MVC isometric contractions separated by 30-s resting periods (5). While discrepancies between studies might originate from differences in the tested muscles or in the experimental methods, we suggest that the characteristics of the fatiguing task are mainly responsible. It is well known that fatigue is “task-dependent” (8) and parameters such as contraction mode, total contraction time, contraction intensity and sustained vs repeated contractions may all influence the level of fatigue, as well as its etiology. This is, for example, supported by the longer time needed to recover from intermittent submaximal contractions compared to sustained contractions (23, 29), and we suggest this applies as well for muscle stiffness. Therefore, and in opposition to the present results and unequivocal decreased stiffness after dynamic (6) or intermittent contractions (7), the use of prolonged isometric contractions may not lead to stiffness modification as suggested by findings of Hunter et al. (4)

and Portero et al. (5). This is further supported by the recent study of Bouillard et al. (17) that reported no changes in shear modulus recordings for a given force level after a mean 227 s sustained 40% MVC of the ADM muscle.

Even though the present results suggest that intrinsic active muscle stiffness is altered by fatigue, they do not provide direct information about the potential physiological mechanisms involved. At the fiber level, active muscle stiffness is closely linked to the number of attached cross bridges (16). If this was strictly the case, then muscle shear modulus should theoretically remain the same for a given absolute muscle force, even in the presence of fatigue, as observed by Bouillard et al. (17). In the present study, shear modulus was clearly decreased at the given absolute force levels for both VL and ADM muscles, as indicated by K_{30} and K_{40} decreases, as well as the slopes of the relationships between shear modulus and absolute joint force. This would suggest that muscle fiber stiffness, and by extension active muscle stiffness as estimated by shear modulus, may not be a direct measure of the number of force-generating heads of myosin (30). Alternatively, it has recently been suggested that stiffness at the sarcomere level is related to the myofilaments mechanical properties themselves (31–33). As such, several studies have demonstrated that the attached myosin heads include both the force-generating and the non-force-generating heads (31, 34), with the latter contributing to the stiffness but not to force generation. Moreover, the myosin powerstroke does not only produce force in the axis of shortening but a radial component also exists (*i.e.*, orthogonal to the long axis of the myofilaments) (35). This radial component varies with the lattice spacing (*i.e.*, the radial spacing between the contractile filaments), which is influenced by the osmotic pressure as a result of metabolic by product accumulation during repeated contractions (36). Taken together, this suggests that the ratio of force to stiffness per cross-bridge may vary in the presence of fatigue

induced by repeated contractions. Further studies are needed to bring new insights into the molecular mechanisms that may be involved in the decrease in active muscle stiffness due to fatigue.

This study shows that active muscle stiffness decreases after a fatiguing task involving intermittent submaximal isometric contractions. This was observed for both VL and ADM muscles. Further studies should now analyze the mechanisms involved in this fatigue-induced decreased active muscle stiffness and determine how this may affect muscle performance and the risk of injury.

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FIGURE CAPTIONS

Figure 1. Experimental design for testing the *vastus lateralis* (VL) and *abductor digiti minimi* (ADM) muscles. Both sessions followed the same experimental protocol (except for the active stiffness measurement: 10 to 60% of maximal voluntary contraction (MVC) force plateau -VL- vs. 20-s smooth linear force ramp from 0 to 60% of MVC -ADM-). For exercise: ON is the contraction time and OFF is the rest between two contractions.

Figure 2. Typical shear modulus – force relationship obtained pre- (black and grey) and post-fatigue (white) for *vastus lateralis* (A) and *abductor digiti minimi* (B). Regression equations and correlation coefficients are presented for each relationship. Typical examples of shear modulus maps for similar force levels are presented. The colored region represents the shear modulus map, with the warmest color representing the highest shear modulus values. To obtain a representative value, the shear elastic modulus (kPa) was averaged over the greatest muscular area, avoiding aponeurosis.

Figure 3. Shear modulus for a given absolute force level measured pre- and post-fatigue (40 and 30% of the MVC pre-fatigue respectively for *vastus lateralis* –A- and *abductor digiti minimi* –B-). The bar graph represents the mean value and circles represent the individual data.

Figure 4. Active muscle stiffness index pre- and post-fatigue of the *vastus lateralis* (A) and *abductor digiti minimi* (B). The bar graph represents the mean value and circles represent the individual data.

Figure 1

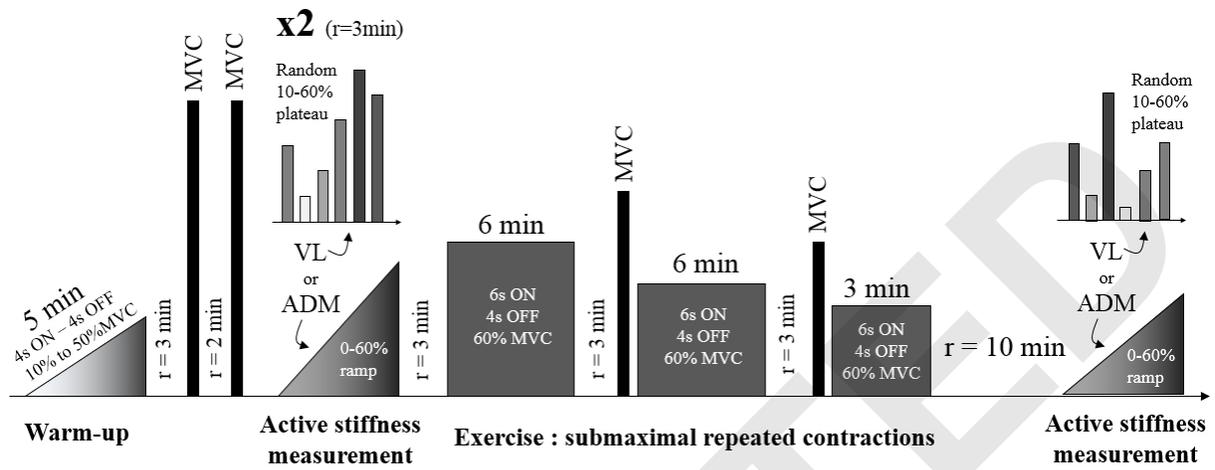


Figure 2

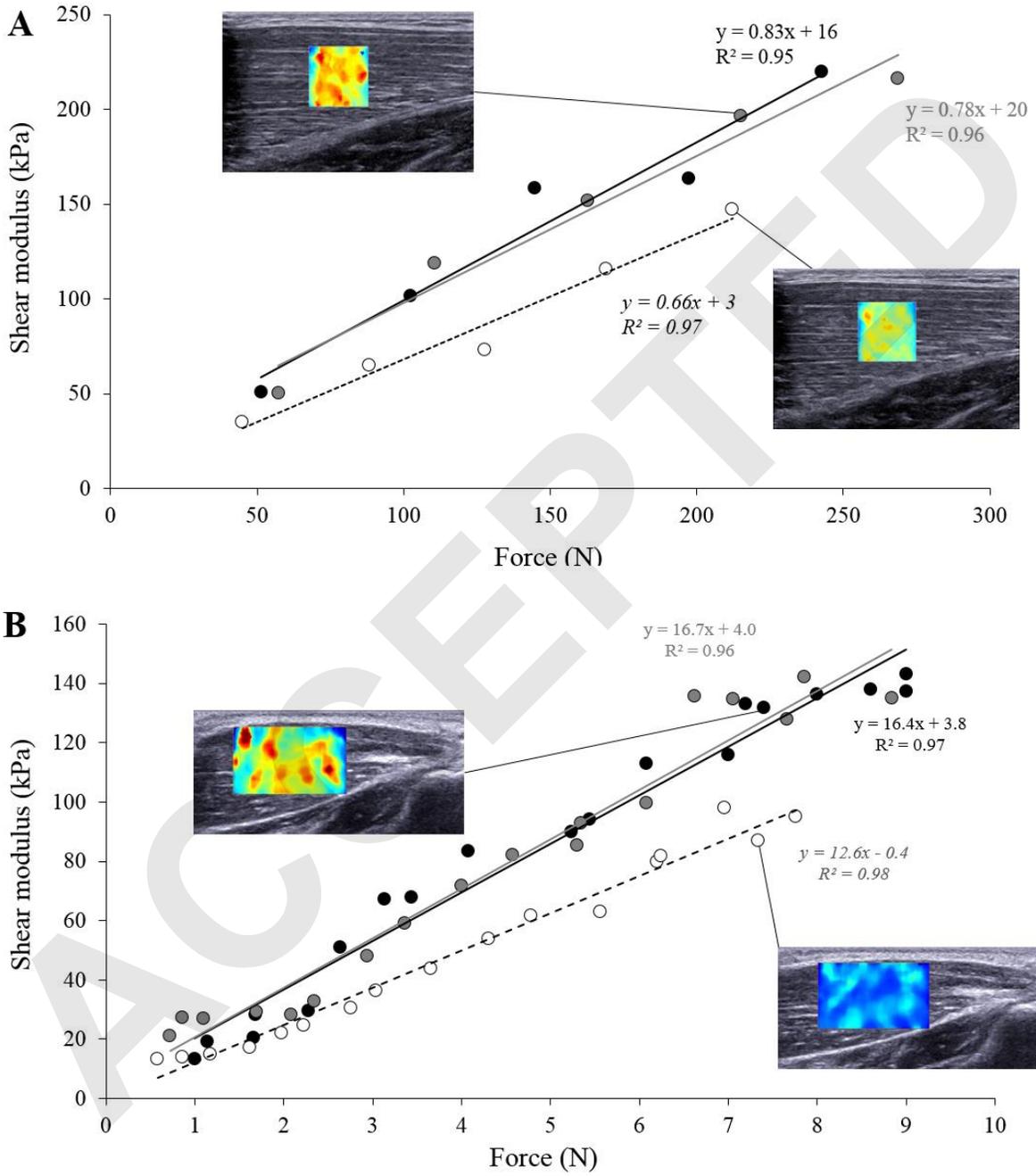


Figure 3

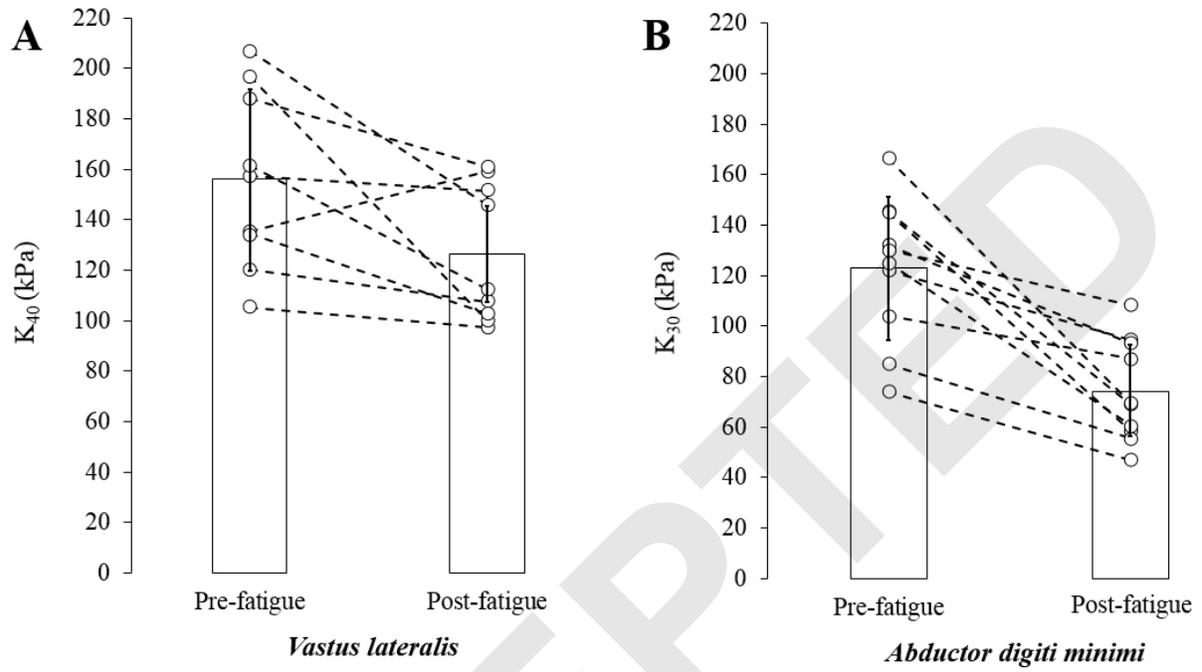


Figure 4

