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CHANGES IN LOCALIZED MECHANICAL PROPERTIES AND EDEMA FOLLOWING EXERCISE-INDUCED MUSCLE DAMAGE

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SUMMARY
The present study aimed (i) to characterize the effect of eccentric exercise on localized muscle mechanical properties and (ii) to determine if potential changes are related to muscle edema. Delayed onset muscular soreness, maximal strength, shear elastic modulus (μ) measured using an ultrasound elastographic technique and T_2 relaxation time were quantified in *biceps brachii* and *brachialis* before, and up to 21 days following eccentric exercise in 12 subjects. μ significantly increased just after the exercise, peaked at 1h while T_2 increased at 48h post-exercise. Such findings exclude edema as the factor responsible for the rise in muscle stiffness following damaging exercise. The greater increases in muscle stiffness observed at long muscle lengths suggest that the changes in the muscle mechanical properties originate from the alterations of the reticulum sarcoplasmic function and Ca^{2+} homeostasis. These perturbations have been demonstrated to induce an increase in passive tension, particularly at long muscle lengths where calcium-dependent activation increases.

INTRODUCTION
Eccentric contractions are well-known as a damaging form of muscular exercise. Muscle damage is recognized to induce functional impairments such as a decrease in maximal strength and joint range of motion, combined with an increase in muscular hardness and soreness sensation in the few days following exercise [1]. On the one hand, human studies focusing on the analysis of passive torque-angle curves showed an increase in muscle stiffness of knee extensor, elbow flexor, wrist extensor or plantar flexor muscles subsequently to eccentric contractions [2]. Some works suggested that these findings could be related to tissue swelling triggered by inflammatory processes and fluid accumulation (i.e., muscle edema) [2]. However, both rises in passive tension and muscle stiffness have been observed in the first moments following eccentric exercise, while edema formation occurs during the next 24 to 48h [1,3]. On the other hand, studies performed on animal models raised alternative hypotheses such as the perturbation of calcium homeostasis that could induce cross-bridge attachment responsible for an increase in passive tension [4]. Thus, the purpose of the present study was to directly investigate the localized changes in mechanical properties of the elbow flexor muscles, and their relation to edema, after exercise-induced muscle damage. We hypothesized an increase in muscle stiffness in the absence of edema, reinforcing alternative interpretations of mechanical alterations in damaged muscle.

METHODS
The study was approved by both the Ethical committee and the French national safety agency. 12 subjects performed a session of 3 sets of 10 isokinetic eccentric contractions of the right elbow flexor muscles at 120°.s^{-1} on a Con-Trex MJ dynamometer (CVHAG, Switzerland). Delayed onset muscular soreness (DOMS) and maximal isometric torque (MVC) at 90° of elbow angle (0° = full extension) of the damaged and contralateral arms were measured before (PRE), 1h, 48h and 21 days following eccentric exercise. An Aixplorer ultrasonic scanner (version 4; Supersonic Imagine, Aix-en-Provence, France), coupled with a linear transducer array (4–15 MHz, SL15-4) was used in supersonic shear imaging (SSI) mode (musculo-skeletal preset) [5]. Assuming a linear elastic behavior, μ was calculated: $\mu = \rho Vs^2$, where ρ is the muscle mass density (1000 kg · m^{-3}) and Vs is the shear wave speed.

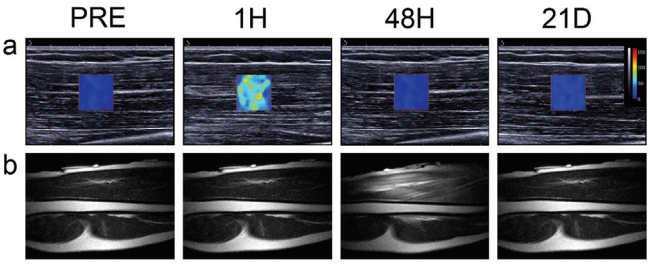

Figure 1. Typical example of elastographic (a) and T_2-weighted MRI images (b) for the *biceps brachii* before (PRE), 1h, 48h and 21 days (21D) post-exercise at a 110° elbow angle. The colored region represents the shear elasticity map (a, scale on the top-right). The white signal observable at 48h (b) indicates the presence of fluid accumulation (edema).

μ was determined in *biceps brachii* (Fig.1a) and *brachialis* muscles at 70°, 110° and 160° of elbow angle on damaged limb and at 110° on contralateral limb. Longitudinal T_2-weighted magnetic resonance images of the damaged arm were obtained at 110° (Fig.1b) using a dedicated low-magnetic field (0.25 Tesla) musculoskeletal MRI system (G-scan, ESAOTE, Genoa, Italy). The MR sequence was as follows: spin echo technique; repetition time/echo time = 3000 ms/20, 50, 85 ms. Marks were placed on the skin to ensure that MRI and elastographic mesurements

were performed at the same locations. Both μ and T_2 measurements were performed on proximal, medial and distal portion of the *biceps brachii* to investigate potential difference between sites.

All data being normally distributed (Kolmogorov-Smirnov test), four separate two-way ANOVAs (time × arm) with repeated measures were applied to DOMS, MVC and μ measurements to attest of the presence of muscle damage. One three-way ANOVA (time × angle × site) was applied to μ values and one two-way ANOVA (time × site) was applied to T_2 values. When the sphericity assumption in repeated measures ANOVAs was violated, a Geisser-Greenhouse correction was used. Post-hoc tests were performed by means of Newman-Keuls procedures. A linear pearson correlation was performed between MVC and μ changes, relatively to PRE value, in order to determine a correlation coefficient (r). The significance level was set at $P < 0.05$. The data are presented as mean ± SE.

RESULTS AND DISCUSSION

DOMS significantly increased at 1h (1.5/10; $P < 0.05$) and peaked at 48h post-exercise (3.8/10; $P < 0.05$). MVC of the damaged arm was reduced at 1h (-29 ± 6%) and 48h (-24 ± 7%) post-exercise compared to contralateral arm ($P < 0.05$), thus confirming the presence of damage following exercise. DOMS and MVC were not significantly different from PRE at 21 days. ANOVAs revealed a time effect on μ and T_2 values at 110° of elbow angle ($P < 0.0001$; Fig.2).

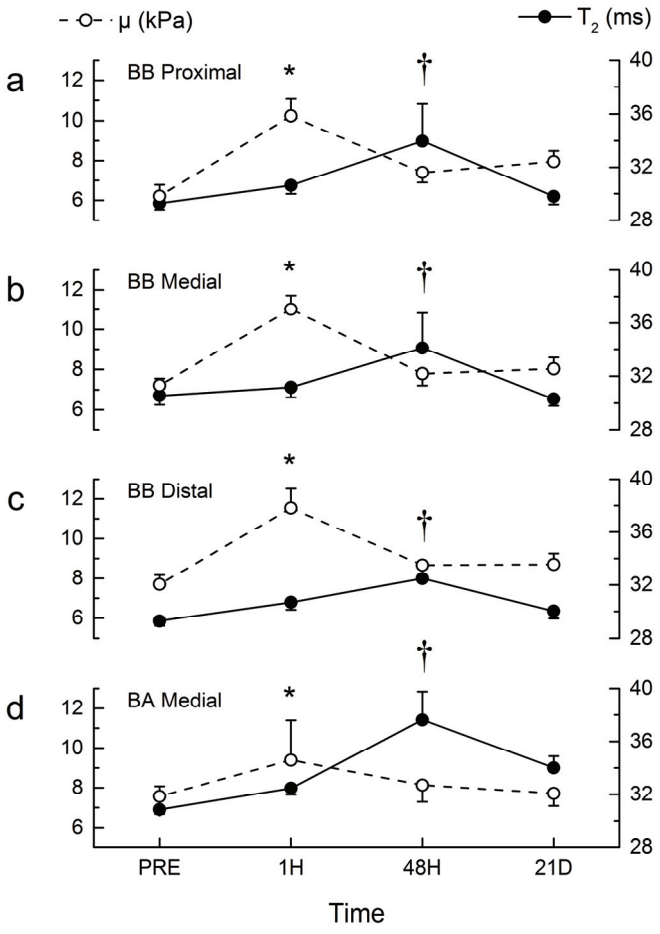

Figure 2. Time-course of shear elastic modulus and T_2 measurements at 110° of elbow angle at proximal (a), medial (b) and distal (c) portion of *biceps brachii* (BB) and *brachialis* (BA, d). *, μ value different from PRE; †, T_2 value different from PRE.

These results are in accordance with a previous study that showed an increase in the shear modulus of the *medial gastrocnemius* following eccentric exercise [6], while the increase is larger in the present study. T_2 was not affected at 1h, but significantly increased at 48h (+14 ± 5%) for both muscles, while μ was similar to PRE measurement at 48h. These dissociated time-courses of shear elastic modulus and edema confirm our hypothesis and constitute the first direct evidence that fluid accumulation (edema) is not the primary factor responsible for the rise in muscle stiffness subsequent to damaging exercise. The three-way ANOVA applied to μ measurements revealed a time × angle interaction, underlying the muscle length-dependency of stiffness modifications ($P = 0.015$). Indeed, while no significant changes were observed at 70° angle ($P = 0.99$), μ increased by 52 ± 9% at 110° and by 80 ± 14% at 160° of elbow angle 1h post-exercise ($P < 0.05$). Recent investigations demonstrated that eccentric exercise results in a depression of sarcoplasmic reticulum function that alters the intra-muscular calcium homeostasis [4]. These perturbations lead to an increase in resting Ca^{2+} levels in damaged fibers. It has been suggested that the rise in Ca^{2+} is sufficient to trigger a low level of activation of muscle fibers and increase passive tension, although there is no direct validation of this hypothesis in humans [2]. Several studies proposed that this sensivity to calcium is length-dependent, with greater activation level at long muscle lengths [4]. Our findings are in agreement with such length-dependent changes in passive mechanical properties of the muscle since shear elastic modulus was not affected at short muscle lengths while it dramatically increased at long muscle lengths (+80%). 1h after exercise, the changes in μ at long muscle length (160°) were significantly correlated to MVC decrease (r = 0.88). Since muscle force impairments are correlated to the number of damaged muscle fibers and consequently to the Ca^{2+} release induced by eccentric exercise [7], this result supports the potential role of Ca^{2+} in the modifications of muscular mechanical properties subsequent to damaging exercise.

CONCLUSIONS

Biceps brachii and *brachialis* localized stiffness increased following eccentric exercise. This immediate rise in passive tension post-exercise is unlikely to be associated with muscle swelling or edema. However, several indicators tend to support that the perturbation of calcium homeostasis subsequent to the structural alterations induced by eccentric contractions plays an important role in the modifications of muscle mechanical properties after damaging exercise.

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REFERENCES

1. Howell J, et al., *J Physiol*. **464**:183-196, 1993.
2. Proske U & Morgan D, *J Physiol*. **537**:333-345, 2001.
3. Chleboun G, et al. *Med Sci Sports Exerc*. **30**:529-535, 1998.
4. Balnave G & Allen D, *J Physiol.* **492**:705-713, 1996
5. Lacourpaille L, et al. *Physiol Meas*. **33**:19-28, 2012.
6. Green M, et al. *NMR Biomed*. **25**:852-8, 2012.