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ABSTRACT

Background: Magnetic resonance elastography (MRE) was performed in healthy and pathological muscles in order to provide quantitative muscle stiffness data to clinicians. Moreover, due to the lack of data on muscle children, the present work studies age-related changes of the mechanical properties from childhood to adulthood.

Methods: 26 healthy subjects composed of 7 children (8-12 years), 9 young adults (24-29 years) and 10 middle-aged adults (53-58 years) underwent a MRE test. Shear modulus (μ) and its spatial distribution, as well as the attenuation coefficient (α) were measured on the vastus medialis muscle at rest and at contracted conditions (10% and 20% of the maximum voluntary contraction) for each group.

Findings: The shear modulus linearly increases with the degree of contraction for young adults while it is maximum at 10 % of the maximum voluntary contraction (MVC) for children ($\mu\_{children\_10\%}$ = 14.9 kPa (SD 2.18)) and middle-aged adults ($\mu\_{middle\text{-}aged\_10\%}$ = 10.42 kPa (SD 1.38)). Mapping of shear modulus revealed a diffuse distribution of colors reflecting differences in muscle contractile properties as a function of age. Moreover, the attenuation coefficient showed a similar behavior for all groups, i.e. a decrease from the relaxed state to 10 % MVC followed by a plateau at 20% MVC.

Interpretation: This study demonstrates that the MRE technique is sensitive enough to detect changes in mechanical properties from childhood to adulthood and has the potential to determine muscle mechanical properties for patients suffering from neuromuscular disorders, particularly during therapeutic trials.

Key words: shear modulus map, age, muscle growth, magnetic resonance elastography

INTRODUCTION

Muscle tissue is strongly utilized throughout life during daily activity, exercise or physical work inducing different muscle efforts. Muscles pathologies such as myopathy detected in childhood, fibromyalgia present in adult muscles or muscle weakness exhibited by older individuals can also alter the muscle stiffness and its morphological properties. Thus, a complete understanding of the morphological and mechanical properties of muscle will allow for the development of muscle prevention.

As a consequence of the morphological changes, the mechanical properties of muscle are also affected. Most of the studies (e.g. Akima et al., 2001; Lynch et al., 1999) used an isokinetic dynamometer to measure peak torque during knee extension and flexion, or concentric and eccentric elbow movements. All of these analyses showed a gradually decrease of the mechanical parameters, from 20 to 85 years of age for men and for women. Since 2001, magnetic resonance elastography (MRE) technique has also been used to characterize *in vivo* mechanical properties of healthy and diseased muscle (Dresner et al., 2001; Basford et al., 2002; Bensamoun et al., 2006, 2007; Brauck et al., 2007). This technique analyzed the propagation of shear waves inside the muscle allowing for the measurement of shear modulus which is related to the muscle function. Indeed, MRE was performed on patients with lower-extremity neuromuscular dysfunction (Basford et al., 2002), hyperthyroidy (Bensamoun et al., 2007) and fibromalgia (Chen et al., 2007, 2008) in order to establish a muscle stiffness data base for the follow up of patient and the effect of treatment. Furthermore, Domire et al. (2009a) have analyzed the standard deviation of the shear modulus to investigate the muscle tissue homogeneity and a relationship was found between the age (from 50 to 70 years of age) and the tissue homogeneity for the tibialis anterior muscle.

In addition to the shear modulus, MRE technique provides a wave attenuation coefficient as a measure of muscle quality (Domire et al., 2009b). Most of muscle MRE studies were performed on adult's muscles, from 20 to 80 years of age, but there is a lack of data characterizing the mechanical properties of muscle for children. This study is the first to show the capability of the MRE technique to measure children muscle stiffness. Morphological properties were widely quantified by ultrasound studies on healthy children in order to be compared with children affected by neuromuscular disorders (Kamala et al., 1985; Heckmatt et al., 1988; Lamminen et al., 1988; Schedel et al., 1992; Schmidt et al., 1993; Zuberi et al., 1999; Pillen et al., 2007).

The purpose of this study is to characterize the vastus medialis mechanical properties at different muscle conditions for children, young adults and middle-aged adults. Moreover, the originality of this present work is to introduce for the first time the evolution of the shear modulus map related to the level of contraction.

MATERIALS AND METHODS

Participants

Seven prepubescent children (6 males and 1 female, mean age = 10.9 yrs (SD 0.6), range = 8-12, mean Body Mass Index: BMI = 17.3 (SD 0.9)), nine young adults (5 males and 4 females, mean age = 26.4 yrs (SD 1.7), range = 24-29, mean BMI = 23.69 (SD 3.34)) and ten middle-aged adults (3 males and 7 females, mean age = 55.2 yrs (SD 2.39), range = 52-58, mean BMI = 26.39 (SD 4.72)) without muscle abnormality and no history of muscle disease underwent a MRE test. These three age points have been chosen to represent different muscle states corresponding to the muscle growing phase (children), a mature muscle (young adult) and the first beginning of aging muscle (middle-aged adults). This study has been approved by the institutional review board and informed consents were obtained from adult participants and from the parents for minor volunteers.

Experimental setup for magnetic resonance elastography (MRE) technique

The subject lays supine on an adult leg press (Bensamoun et al., 2006) which was adapted for children's size. The knee was flexed to 30° with the right foot placed on a footplate, in which a load cell (SCAIME, Annemasse, France) was fixed to record the developed force and a visual feedback (LABVIEW program) of the applied load is given to the volunteers inside the MR room. Then, a pneumatic driver consisting of a remote pressure driver connected to a long hose was wrapped and clamped around the subject's thigh and a custom-made Helmholtz surface coil was placed around the thigh. Shear waves were induced through the thigh muscles at 90Hz (*f*).

MRE experiments were performed for each subject on the VM muscle, in the relaxed and contracted (10% and 20% of the maximum voluntary contraction: MVC) states reflecting the muscular activity of the quadriceps muscle. A delay (few minutes) between individual experiments was given to the volunteers in order to avoid fatigability effects. This procedure was repeated in the same condition, at different times, on few subjects for the different age groups.

Magnetic resonance elastography (MRE) tests

Axial image of the thigh muscle was acquired with a gradient echo sequence (1.5T General Electric HDxt MRI) in order to place an oblique scan plane on the medial side of the thigh to visualize the vastus medialis muscle. Then, MRE images were collected with a 256 x 64 acquisition matrix (interpolated to 256 x 256), a flip angle of 45°, a 24 cm field of view and a slice thickness of 5 mm. Four offsets were recorded with the vastus medialis (VM) muscle in relaxed and contracted (10%, 20% MVC) states during a scan time of 52 seconds using a TR/TE of 100 ms/23 ms.

Image processing and data analysis

MRE technique provides anatomical image of the VM muscle (Fig. 1a) as well as phase image (Fig. 1b) showing the shear wave displacement within the muscle. A white profile was manually placed in the direction of the wave propagation that follows the orientation of the fascicle paths as previously demonstrated (Debernard et al., 2011). The quantification of the wavelength (λ) along this profile leads to the measurement of the local shear modulus ($\mu\_local = \rho\lambda^2 f^2$, with ρ = 1000 kg/m^3 for the muscle density) assuming that the muscle is linearly elastic, locally homogeneous, isotropic and incompressible.

Attenuation coefficient (α) of the shear wave displacement was measured along the prescribed white profile. This parameter is obtained from a Fourier Transform of the distance covered by each pixel during the four offsets (Domire et al., 2009b). The amplitude value of the first temporal harmonic of each pixel was extracted at the driven frequency 90Hz providing the amplitude displacement image (Fig. 1c). Then, a curve representing the shear wave displacement amplitude according to the distance (sampling step corresponding to the pixel size: 0.9mm) was obtained and fitted with an exponential function $Ae^{-(\alpha d)}$, with the parameters "A" and "d" representing the displacement amplitude value and the distance along the profile, respectively (Fig. 2).

A map of the shear modulus was generated from the wave displacement image using the local frequency estimate (LFE) algorithm (Manduca et al., 2001) providing a spatial distribution of the muscle shear modulus. Assuming that muscle tissue is locally homogeneous, an ellipsoid region of interest (ROI) was placed around the prescribed profile in order to quantify a more important muscle stiffness area reflected by a global shear modulus (μ_{ROI}) compared to the local one (μ_{local}) measured along the profile.

Statistical analysis

The statistical analysis was performed with the software Statgraphics 5.0 (Sigma Plus, Maryland, USA) using a two-factor ANOVA (age, muscle state) and when P values were significant post hoc t-test (Student-Newman-Keuls) were done. The effects of age and muscle state were determined on shear modulus and attenuation coefficient. The significance was fixed to $P < 0.05$.

RESULTS

Effects of muscle condition (relaxed or contracted) on shear modulus and attenuation coefficient for children, young adults and middle-aged adults.

Young adults showed a significant (*P*<0.01) linear increase of the shear modulus from the relaxed (μ_{young} = 3.83 kPa (SD 0.24)) to 20% MVC (μ_{young} = 12.97 kPa (SD 0.87)), whereas the children and older individuals showed only a significant (*P*<0.01) increase between the relaxed ($\mu_{children}$ = 2.99 kPa (SD 0.19), $\mu_{middle-aged}$ = 3.84 kPa (SD 0.42)) and 10% MVC ($\mu_{children}$ = 14.90 kPa (SD 2.18), $\mu_{middle-aged}$ = 10.42 kPa (SD 1.38)) (Fig. 3a, 3b). We note that children revealed the highest increase of shear modulus (5 fold) from the relaxed to the contracted (10% MVC) state (Fig. 4a), whereas the young and middle-aged adults showed a lower increase of muscle shear modulus (about 2 fold) (Fig. 4b, Fig. 4c). Between 10% and 20% MVC, individuals in their fifties revealed a slightly decrease ($\mu_{middle-aged\_10\%\ MVC}$ = 10.42 kPa (SD 1.38), $\mu_{middle-aged\_20\%\ MVC}$ = 9.13 kPa (SD 1.19)) (Fig. 4c), compared to the young adults which continue to increase their shear modulus with the same ratio (2 fold) (Fig. 4b).

The reproducibility of the shear modulus was measured as the standard deviation (SD) from the repeated MRE tests. The SD measurements were ± 0.32 kPa and 0.45 kPa in relaxed and contracted conditions for all groups, respectively.

The effect of the muscle condition (relaxed or contracted) on the shear wave attenuation was the same for the three age groups. Thus, children, young and middle-aged adults showed a significant (*P*<0.05) decrease of 34.72 m^{-1} (SD 3.13), 18.75 m^{-1} (SD 0.93) and 12.00 m^{-1} (SD 1.64) between the relaxed state and 10% of MVC, respectively (Fig. 5a-5b). In agreement with the shear modulus results, children showed the most important attenuation from the relaxed to contracted (10% MVC) condition. Then, the attenuation coefficient is consistently maintained from 10% to 20% of MVC for all the groups.

Effect of age on the muscle shear modulus and attenuation coefficient for the relaxed and contracted positions

In a relaxed state, the children had a slightly (*P*<0.01) lower shear modulus ($\mu\_{children}$ = 2.99 kPa (SD 0.19)) compared to adult's groups which have similar shear modulus (μ_{young} = 3.83 kPa (SD 0.24), $\mu\_{middle\text{-}aged}$ = 3.84 kPa (SD 0.42)) (Fig. 3b).

At 10% of MVC, the highest significant difference (*P*<0.05) of shear modulus was found between the children and young adults (about 7.6 kPa) while a smaller difference (*P*< 0.01) was found between the adult's groups (about 3 kPa). However, no difference was found between children and middle-aged adults, probably due to the large range of shear modulus measured for children. At 20% of MVC, only a significant (*P*<0.01) difference of shear modulus was found between adult's groups.

The only effect of age on the shear wave attenuation was found when the muscle was relaxed (Fig. 5a). Indeed, children revealed a significant (*P*<0.05) difference with young adults (about 13 m^{-1}) (Fig. 5a). The results demonstrate any effect of age on the shear wave attenuation when the muscle was contracted.

Cartography of stiffness

Figure 6 illustrates the shear modulus map and the corresponding anatomical image obtained for the children, young and middle-aged subjects when the VM muscle was at rest and at contracted positions (10% and 20% of MVC). At rest, the shear modulus map for the three groups showed a homogeneous purple color in the region of interest (ROI) (Fig. 6a, Fig. 6d, Fig. 6g) placed around the red profile where the shear modulus was locally quantified.

The comparison of the shear modulus measured inside the ROI ($\mu_{children}$ = 3.61 kPa (SD 0.27), μ_{young} = 4.14 kPa (SD 0.26), $\mu_{middle\text{-}aged}$ = 3.2 kPa (SD 0.48)) and along the red profile ($\mu_{children}$ = 2.99 kPa (SD 0.19), μ_{young} = 3.83 kPa (SD 0.24), $\mu_{middle\text{-}aged}$ = 3.84 kPa (SD 0.42)) was in the same range.

When the VM muscle was contracted, the shear modulus map exhibited a diffuse distribution of colors, around the region of interest (Fig. 6b-6c, Fig. 6e-6f, Fig. 6h-6i), demonstrating that the shear waves were sensitive to the muscle media and more specifically to the contraction of the muscle fibers. At 10% MVC, children showed the highest shear modulus value locally measured along the profile and is well represented on the shear modulus map (μ_{local} = 14.9 (SD 2.02) vs μ_{ROI} = 12.4 (SD 1.36)) with a distribution of stiffer tissues inside the ROI (Fig. 6b) in response to the muscle contraction. For the same level of contraction (10% MVC) stiffer muscle tissue showed up inside the ROI for children (Fig 6b) (μ_{ROI} = 12.4 (SD 1.36)) compared to adult groups (μ_{ROI} = 9.08 (SD 2.29)) (Fig. 6e-6h). Moreover, this result is in agreement with the highest shear modulus measured locally along the white profile for children (μ_{local} = 14.9 kPa (SD 2.02)) compared to adult groups (μ_{local} = 8.88 kPa (SD 2.18)). At 20% MVC, young adults (Fig.6f) exhibited stiffer muscle tissue around the region of interest (μ_{ROI} = 11.9 kPa (SD 1.38)) compared to the mapping obtained at 10% MVC (μ_{ROI} = 7.46 kPa (SD 0.85)) (Fig. 6e). This result is in agreement with the linear increase of shear modulus previously found between 10% (μ_{local} = 7.33 kPa (SD 1.23) and 20% MVC (μ_{local} = 12.97 kPa (SD 0.87)). At the opposite, middle-aged participants showed a mapping with less stiff tissue around the ROI at 20% MVC (μ_{ROI} = 8.53 kPa (SD 2.01)) (Fig. 6i) than 10% MVC (μ_{ROI} = 10.7 kPa (SD 2.06)) (Fig 6h), confirming the slight decrease of the shear modulus locally measured at 10% MVC (μ_{local} = 10.42 kPa (SD 1.38) and at 20% MVC (μ_{local} = 9.12 kPa (SD 1.19)).

DISCUSSION

This study is the first to measure muscle shear modulus in children through the use of MRE technique and to study changes of the mechanical properties from childhood to adulthood.

The increase of the muscle shear modulus with the level of contraction is in agreement with the literature (Dresner et al., 2001; Basford et al., 2002; Bensamoun et al., 2006). Slight differences in values may exist due to either the experimental set up (pneumatic vs. mechanical driver) or the choice of frequency (90Hz vs. 120Hz). The originality of the present study was to show the evolution of the shear modulus map related to the level of contraction. Indeed, the mappings obtained at rest, 10% MVC and 20% MVC provided information about the spatial distribution of the contracted muscle area related to the physiological activity or other intrinsic muscle parameters such as the degree of anisotropy, the pre-stretch of muscle fibers or connective tissue as well as the boundary conditions. Stiffnesses's images were also presented by Chen at al. (2007, 2008) for upper trapezius muscle affected by myofascial pain in order to visualize and to localize areas of higher stiffness.

The behaviors of the muscle contractile properties were found to be different as a function of age. At 10% MVC, individuals in their fifties as well as children have already reached a maximal shear modulus compared to young adults that realize progressive muscle fibers recruitment according to the degree of contraction (Bensamoun et al., 2006). Indeed, the present study revealed that persons in their fifties required a more important recruitment of fast fibers to reach a small level of contraction (10% MVC). Thus, the massive recruitment of fibers may explain the difference (about 3kPa) in shear modulus between young and middle-aged subjects. However, at a higher level of contraction (20% MVC), middle-aged subjects exhibited a slight decrease of muscle stiffness that may be due to changes in muscle structure (decline of the size and number of fibers) and physiology (diminution of myosin protein).

Children showed a significant lower shear modulus at rest compared to the adult groups which have similar muscle stiffness. This result demonstrated the capability of the shear wave to reflect different age-related muscle media. According to the present results, it is apparent that muscle structure is different during the childhood, while it is the same between 20 and 59 years of age. Similar results were found by Domire et al. (2009a), i.e. no significant difference of shear modulus measured at rest for the tibialis anterior muscle between 50 and 70 years of age. This last result is surprising because aging muscle is well present at 70 years of age but the effect of age may not be the same for all kinds of skeletal muscles and a larger range of shear modulus may be found for oldest subjects depending of the physical condition of the person at this age. For a small level of contraction (10% MVC) children demonstrated the highest shear modulus values compared to adult groups. Indeed, during the growing muscle process there is an on-going structural organization with the lengthening of the fibers, leading to random and uncontrolled fibers recruitment (Debernard et al. 2011). As a consequence, childhood muscle was unable to control progressively muscle fibers recruitment to perform a small contraction, and instead contract unintentionally all the muscles fibers leading to a high shear modulus. Over activation of the muscle at low activities (< 25% of MVC) was also found in the literature for the triceps surae muscle of children (7 to 11 years old) (Grosset et al., 2008).

The attenuation coefficient is a mechanical parameter reflecting the composition of the muscle media. Attenuation coefficients obtained in this study for relaxed muscle were in the same range as those found in the literature on the same muscle (Domire et al., 2009b). To our knowledge, the present work showed the first attenuation coefficient data base for the contracted state of muscle. According to the present results, it can be stated that the attenuation coefficient revealed the muscle condition, with a decrease of this parameter during muscle fibers stretching. Indeed, this last result could be used by clinicians to better characterize neuromuscular diseases.

CONCLUSION

In conclusion, this study demonstrates that MRE technique is sensitive enough to detect changes in contractile properties from childhood to adulthood**,** and it would be of interest to extend this study at different age points in order to accurately follow changes occurring during the growing muscle phase and aging process. Furthermore, the characterization of the mechanical properties for normal children will provide muscle stiffness database to clinicians in order to quantitatively assess pathological muscles and the effects of treatments and therapies.

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Figure Legends:

Fig. 1: A: anatomical image of the vastus medialis (VM) muscle in a relaxed state. B: Phase image with a white profile drawn along the direction of the shear wave's propagation following the path of muscle fascicle. C: Amplitude image resulting from the amplitude value of the first temporal harmonic extracted at the driven frequency 90Hz along each pixel.

Sr: Sartorius; VM: Vastus Medialis.

Fig. 2: Computation of the attenuation coefficient α (m^{-1}) from the wave displacement amplitude in function of the distance.

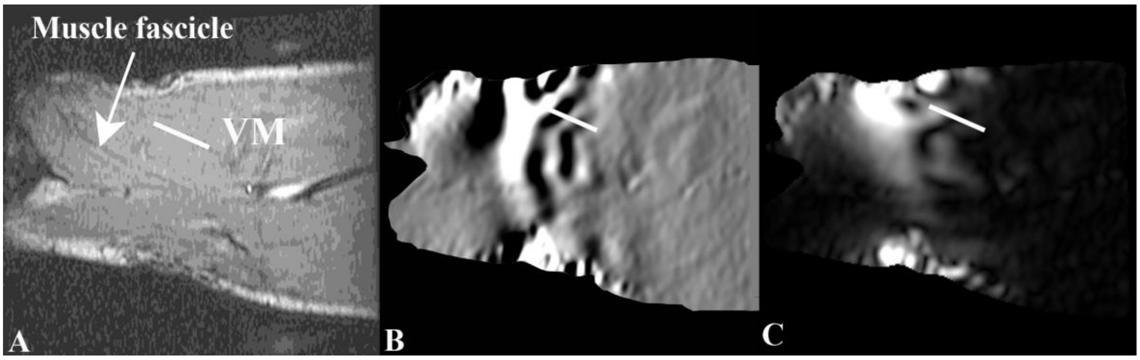
Fig. 3: Bar graph (A) with the corresponding data (B) of the local shear modulus ($\mu_{\_local}$) measured along the propagation of the shear waves for each age points and for different VM muscle conditions (rest, 10% MVC, 20% MVC). The table (B) showed the comparison of the shear modulus measured locally ($\mu_{\_local}$) and inside the region of interest ($\mu_{\_ROI}$). (** $P < 0.05$ and *$P < 0.1$).

Fig. 4: Individual bar graph illustrating the VM shear modulus in relaxed and contracted (10% and 20% MVC) conditions for children (A), young adults (B) and middle-aged adults (C) (** $P < 0.05$ and *$P < 0.1$).

Fig. 5: Bar graph (A) with the corresponding table (B) illustrating the attenuation coefficient (α) in relaxed and contracted (10% and 20% MVC) states for children, young and middle-aged adults (** $P < 0.05$ and *$P < 0.1$).

Fig. 6: Mapping of shear modulus for the VM at rest and contracted (10% and 20% of MVC) conditions for the children, young and middle-aged adults. Muscle stiffness was analysed inside the prescribed region of interest (ROI), placed around the red profil for each group and each muscle state.

Figures:



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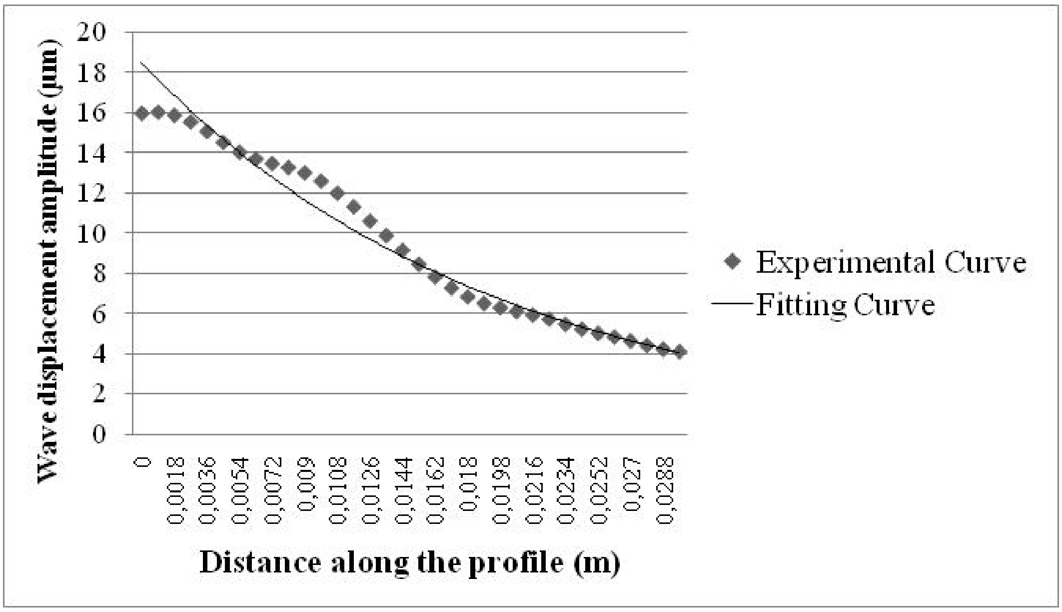

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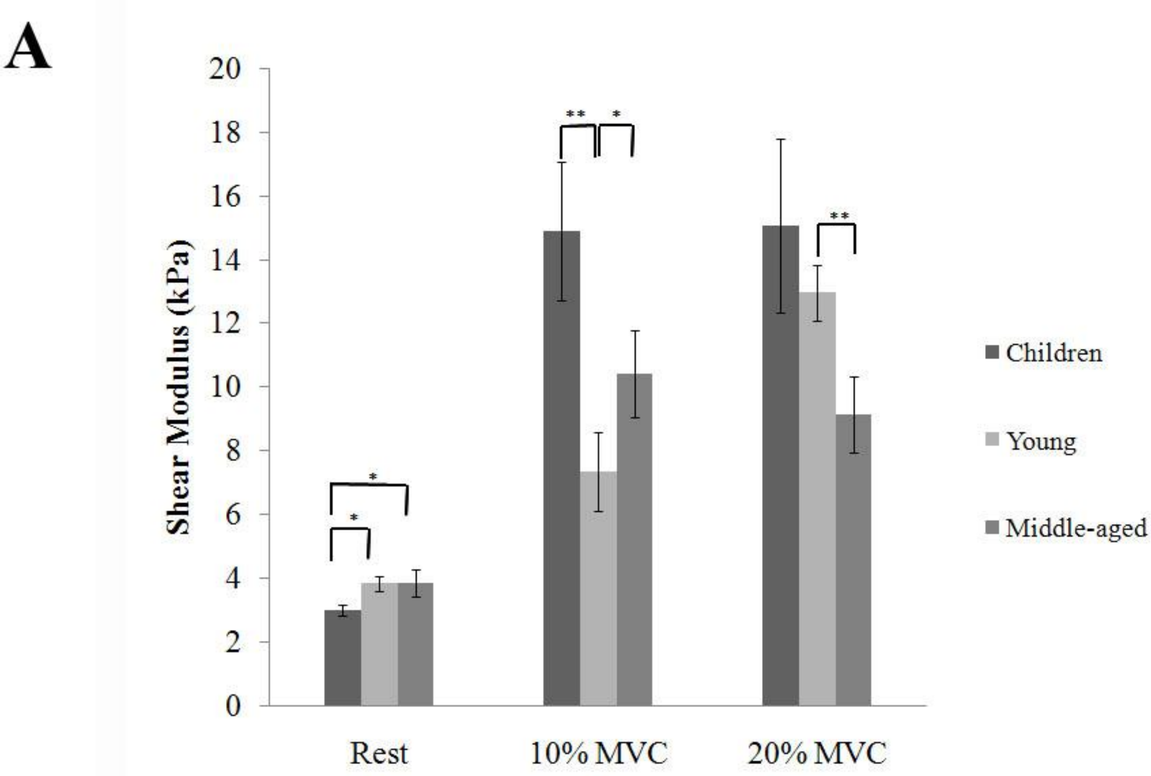


B

	REST		10%MVC		20%MVC	
	μ_Local (kPa)	μ_ROI (kPa)	μ_Local (kPa)	μ_ROI (kPa)	μ_Local (kPa)	μ_ROI (kPa)
Children	2.99 ± 0.19	3.61 ± 0.27	14.9 ± 2.18	12.4 ± 1.36	15.05 ± 2.73	13.3 ± 2.03
Young Adults	3.83 ± 0.24	4.14 ± 0.26	7.33 ± 1.23	7.46 ± 0.85	12.97 ± 0.87	11.9 ± 1.38
Middle-aged adults	3.84 ± 0.42	3.2 ± 0.48	10.42 ± 1.38	10.7 ± 2.08	9.12 ± 1.19	8.53 ± 2.01

Fig. 3: Bar graph (A) with the corresponding data (B) of the local shear modulus ($\mu_{\_local}$) measured along the propagation of the shear waves for each age points and for different VM muscle conditions (rest, 10% MVC, 20% MVC). The table (B) showed the comparison of the shear modulus measured locally ($\mu_{\_local}$) and inside the region of interest ($\mu_{\_ROI}$). (** $P < 0.05$ and *$P < 0.1$).

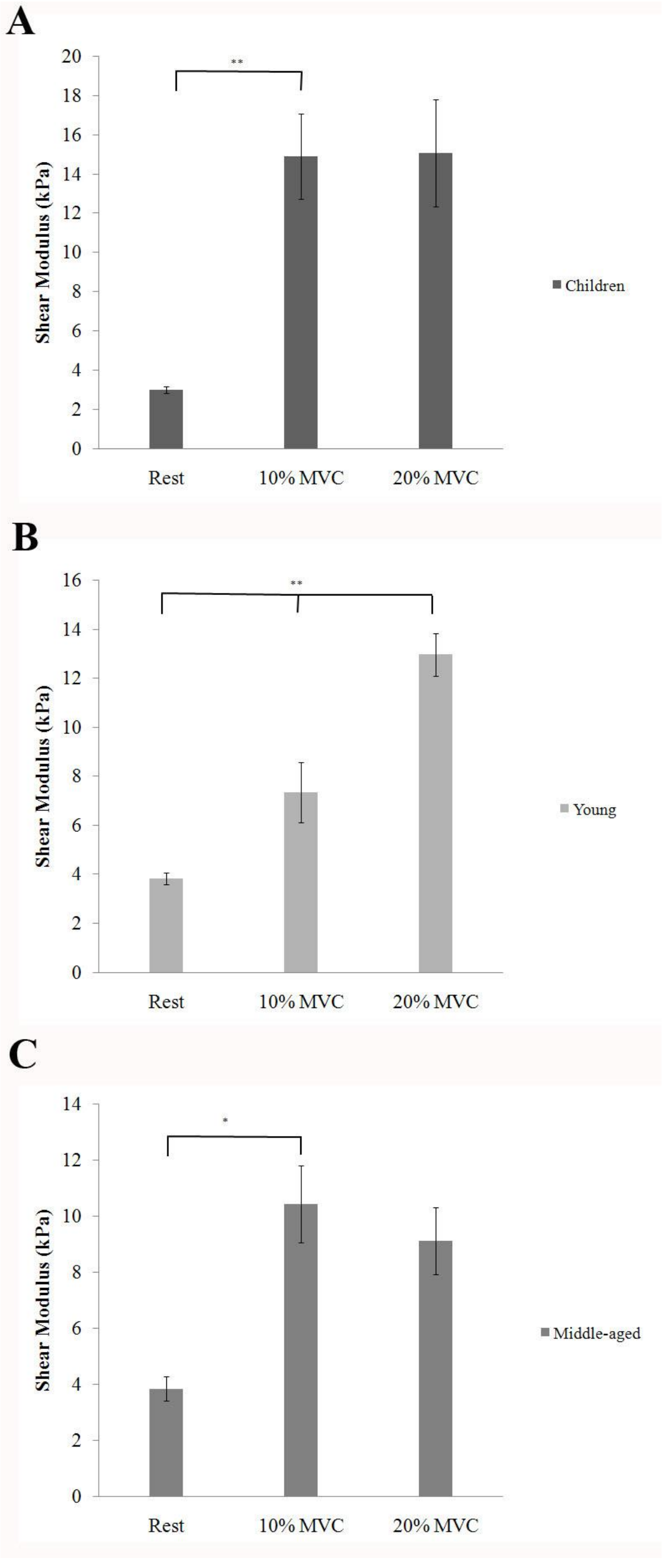
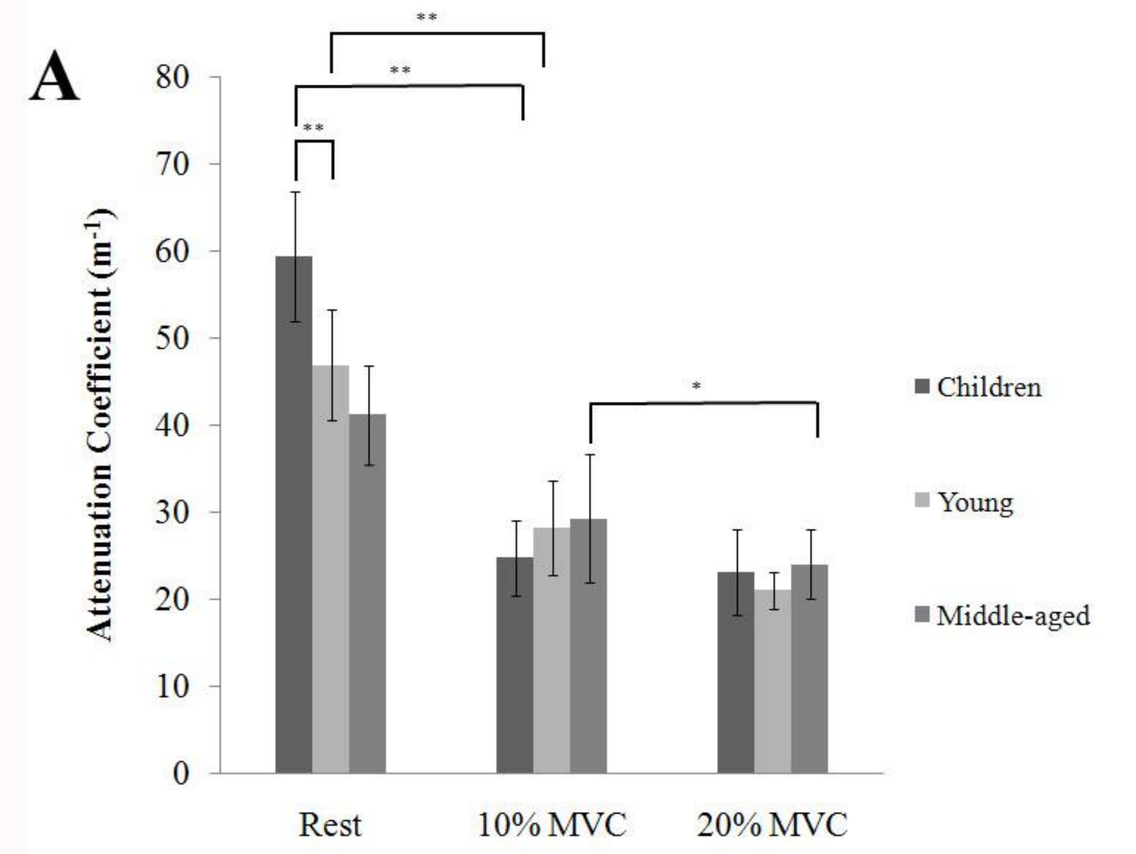


Fig. 4: Individual bar graph illustrating the VM shear modulus in relaxed and contracted (10% and 20% MVC) conditions for children (A), young adults (B) and middle-aged adults (C) (** $P < 0.05$ and *$P < 0.1$).



B

	Attenuation coefficient (m^{-1})		
	REST	10%MVC	20%MVC
Children	59.43 ± 7.44	24.71 ± 4.31	23.17 ± 4.91
Young Adults	46.89 ± 6.35	28.14 ± 5.42	21 ± 2.13
Middle-aged adults	41.2 ± 5.68	29.2 ± 7.37	24 ± 3.93

Fig. 5: Bar graph (A) with the corresponding table (B) illustrating the attenuation coefficient (α) in relaxed and contracted (10% and 20% MVC) states for children, young and middle-aged adults (** $P < 0.05$ and *$P < 0.1$).

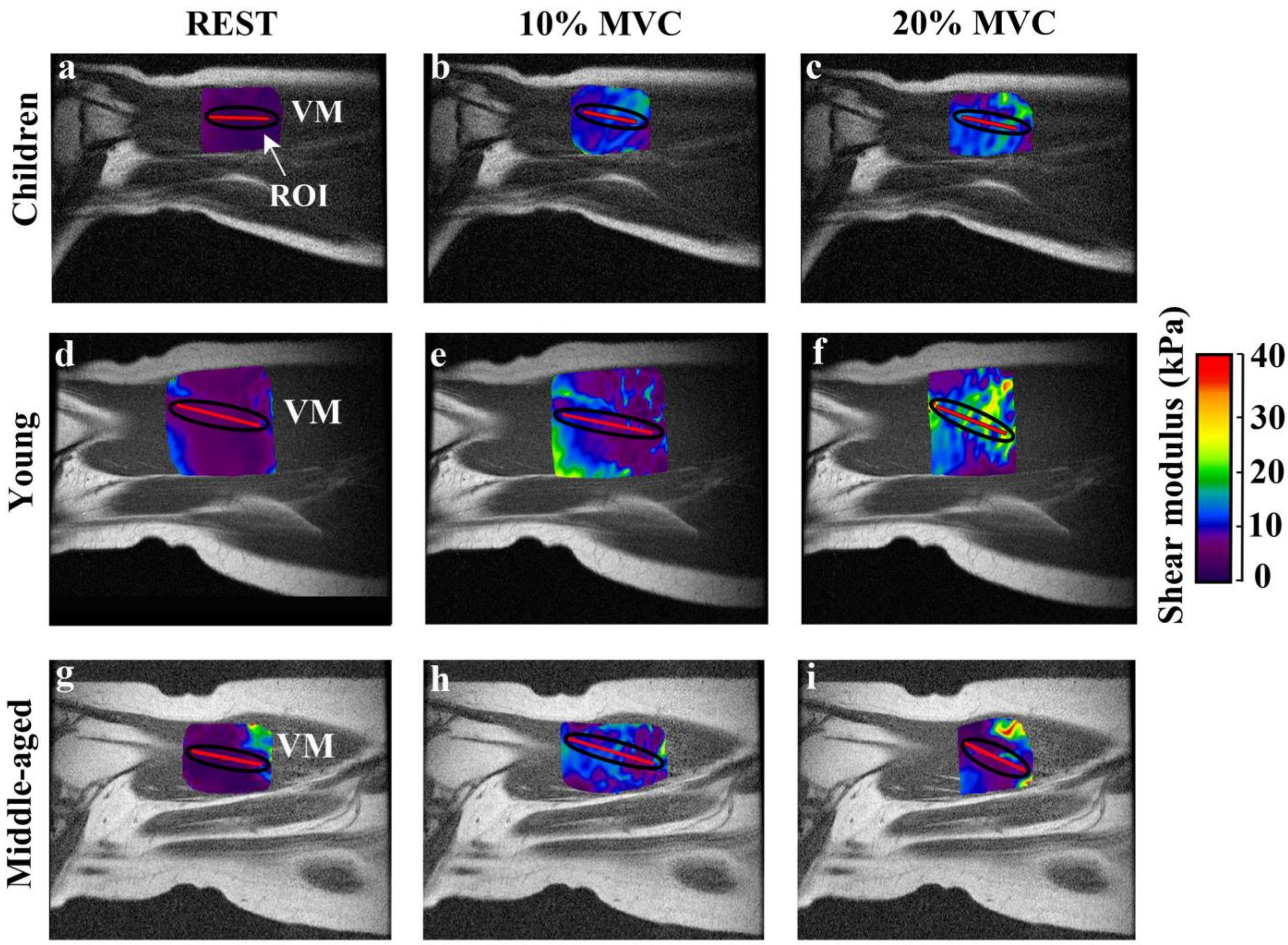


Fig. 6: Mapping of shear modulus for the VM at rest and contracted (10% and 20% of MVC) conditions for the children, young and middle-aged adults. Muscle stiffness was analysed inside the prescribed region of interest (ROI), placed around the red profil for each group and each muscle state.