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# **Characterization of muscle architecture in children and adults using magnetic resonance elastography and ultrasound techniques**

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## Abstract

The purpose of this study is to characterize the muscle architecture of children and adults using magnetic resonance elastography and ultrasound techniques. Five children (8-12yrs) and seven adults (24-58yrs) underwent both tests on the Vastus Medialis muscle at relaxed and contracted (10% and 20% of MVC) states. Longitudinal ultrasonic images were performed in the same area as the phase image showing the shear wave's propagation. Two geometrical parameters were defined: the wave angle ( $\alpha_{\text{MRE}}$ ) corresponding to the shear wave propagation and the fascicle angle ( $\alpha_{\text{US}}$ ) tracking the path of fascicles. Moreover, shear modulus was measured at different localizations within the muscle and in the subcutaneous adipose tissue.

The association of both techniques demonstrates that the shear wave propagation follows the muscle fascicles path, reflecting the internal muscle architecture. At rest, ultrasound images revealed waves propagating parallel to the children fascicle while adults showed oblique waves corresponding to already oriented ( $\alpha_{\text{US}} = 15.4 \pm 2.54^\circ$ ) muscle fascicles. In contraction, the waves' propagation were in an oblique direction for children ( $\alpha_{\text{US}_{10\% \text{MVC}}} = 10.6 \pm 2.27^\circ$ ,  $\alpha_{\text{US}_{20\% \text{MVC}}} = 10.2 \pm 2.29^\circ$ ) as well as adults ( $\alpha_{\text{US}_{10\% \text{MVC}}} = 15.4 \pm 2.54^\circ$ ,  $\alpha_{\text{US}_{20\% \text{MVC}}} = 17.2 \pm 2.44^\circ$ ). A stiffness variation (1 kPa) was found between the upper and lower parts of the adult VM muscle and a lower stiffness ( $1.85 \pm 0.17$  kPa) was measured in the subcutaneous adipose tissue.

This study demonstrates the feasibility of the MRE technique to provide geometrical insights from the children and adults muscles and to characterize different physiological media.

**Key words** (5): shear modulus, morphological properties, muscle, ultrasound, MRE

## INTRODUCTION

Skeletal muscle tissue is a biological material that has the capacity to adapt its internal architecture to applied stresses. Knowing that muscle morphology is strongly correlated to the produced activity, a better characterization of the muscle structure will help to understand the different mechanisms implicated in muscle injury, age and neuromuscular disorders. The anatomical structure of muscle was first investigated on cadaveric specimens and then with imaging techniques.

Currently, ultrasound is the most widespread method used to quantitatively estimate the muscle architectural characteristics which are represented by the muscle thickness (Kubo et al., 2003), the subcutaneous fat thickness (Heckmatt et al., 1988), the pennation angle (Kubo et al., 2003) and the fascicle length (Kubo et al., 2003). Kawakami et al. (1993) have showed a strong correlation between the muscle fibers pennation and the muscle thickness, while no correlation was obtained with the fiber size (Henriksson-Larsen et al., 1992) and the isometric force developed by the muscle (Rutherford et al., 1992). In addition to the structural parameters, ultrasound imaging can also characterize the muscle aspect with the measurement of the echogenicity (pixel density) and the inhomogeneity obtained as a difference of grey levels (Maurits et al., 2004). Architectural parameters were measured on healthy children (Scholten et al., 2003) and adults (from 20 to 85 years old) (Kubo et al., 2003) in order to establish a data base of normal muscle which will be of use to identify muscle disease, to follow the effects of the treatment and therapy as well as the effects of age (Kubo et al., 2003; Young et al., 1984-1985) and gender (Sipilä et al., 1991-1993) on muscle structure. Since muscle is an active tissue, it is also necessary to characterize the structural parameters when the muscle is contracted. Most of the studies used isometric strength at different knee joint angles using dynamometer device (Fukunaga et al., 1997) or electromyography technique

(Sipilä et al., 1991) so as to measure the ultrasonographic parameters during the contraction. Hodges et al. (2003) showed that the muscle fiber pennation angles and fascicle lengths varied from the relaxed to the isometric state, emphasized by the level of contraction.

In addition to ultrasound, magnetic resonance imaging (MRI) has been used to mainly characterize the muscle volume (Tracy et al., 2003; Morse et al., 2007). MRI sequences (Lund et al., 2002) and segmentation methods (Nordez et al., 2009) were optimized to accurately define this parameter, which is used in theoretical models to simulate the musculoskeletal system (Erdemir et al., 2007). MRI and ultrasound are complementary techniques which are both capable of measuring muscle volume (Walton et al., 1997). Indeed, the largest field of view obtained with MRI allowed the measurement of several muscles volume in one acquisition while it did not allow an accurate visualization of the muscle fascicles which were clearly observed with ultrasound technique. Additional architectural characteristics of muscle were obtained with magnetic resonance elastography (MRE) technique. Indeed, this method generates shear waves inside the muscle tissue in order to measure the mechanical properties (Dresner et al., 2001; Basford et al., 2002; Bensamoun et al., 2006-2007; Brauck et al., 2007; Chen et al., 2007-2008; Domire et al., 2009) and several studies have observed the propagation of the shear waves along the muscle fiber (Bensamoun et al., 2006; Sack et al., 2002). Thus, V-waves, oblique waves and longitudinal waves were observed inside the biceps brachii, the vastus medialis and the sartorius muscles, respectively, reflecting their own muscles architecture.

The originality of this study is to show the feasibility of the MRE technique to provide geometrical insights from the vastus medialis muscle of children and adults. Thus, the purpose is to demonstrate that the propagation of the shear waves follow the muscle fascicle architecture and to reveal the physiological composition of the media. MRI anatomical

structure will be cross-validated with ultrasound imaging when the muscle is in relaxed and contracted conditions.

## **MATERIALS AND METHODS**

### **Participants**

Five children (3 boys and 2 girls, mean age =  $10.6 \pm 0.9$ , range = 8-12, mean BMI =  $16.44 \pm 1.28$ , BMI: Body Mass Index) and seven adults (3 males and 4 females, mean age =  $30.6 \pm 12.2$ , range = 23-58, mean BMI =  $24.43 \pm 1.84$ ) without muscle abnormality and no history of muscle disease underwent a magnetic resonance elastography (MRE) and ultrasound tests. This study has been approved by the institutional review board and informed consents were obtained.

### **Experimental Setup for Magnetic Resonance Elastography (MRE) technique**

The subject lays supine, inside a 1.5T General Electric HDxt MRI machine, on an adult leg press (Bensamoun et al., 2006) which was adapted for children's size. The knee was flexed to  $30^\circ$  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. A pneumatic driver consisting of a remote pressure driver connected to a long hose was wrapped two times and clamped around the subject's thigh one third of the distance from the patellar tendon to the greater trochanter, and a custom-made Helmholtz surface coil was placed around the thigh. Shear waves were induced through the thigh muscles at 90Hz (*f*).

MRE images were collected in a specific orientation of the scout image previously established (Bensamoun et al. 2006) in order to obtain the best plan to track the propagation of the shear waves inside the vastus medialis muscle. Thus, a sagittal plane was selected using a gradient echo technique, a 256x64 acquisition matrix (interpolated to 256 x 256), a flip angle of  $45^\circ$  and a 24cm field of view. Four offsets were recorded with the Vastus Medialis

(VM) muscle stiffness in relaxed and contracted (10%, 20% of the maximum voluntary contraction: MVC) states. The scan time was 52 seconds using a TR/TE of 100 ms/23 ms.

### **MRE Image Processing and Data Analysis**

MRE technique provides anatomical image of the tissue as well as phase image (Fig. 1a) showing the shear wave displacement within the soft tissue. A white linear profile (P1) was visually placed (Ringleb et al. 2007, Chen et al. 2007), with an accuracy of  $\pm 5^\circ$ , in the direction of the wave propagation allowing for the measurement of the wave angle ( $\alpha_{\text{MRE}}$ ) on the Vastus Medialis (VM) muscle at relaxed and contracted conditions for each subject. The wave angle ( $\alpha_{\text{MRE}}$ ) was defined as the angle between the subcutaneous adipose tissue-muscle interface and the direction of the wave propagation.

From the phase image, the wavelength ( $\lambda$ ) was manually measured leading to the computation of the shear modulus ( $\mu = \rho \lambda^2 f^2$ , with  $\rho = 1000 \text{ kg/m}^3$  for the muscle density) assuming that the muscle is linearly elastic, locally homogeneous, isotropic and incompressible. Another profile (P2) was added close to the VM aponeurosis of adults (Fig. 1a) and children (Fig. 1b) to measure the shear modulus at different localizations. In addition, a third profile (P3) was placed within the subcutaneous adipose tissue in order to show the sensibility of the shear wave to the composition of the media.

### **Experimental Setup for Ultrasound (US) Imaging**

The subject lays supine on the same leg press with the same position (knee at  $30^\circ$ ) as for the MRE test to image the shear wave displacement (Fig. 2a) and the corresponding ultrasonic image (Fig. 2b) performed with B-mode (Logic E9, GE, Velizy, France). To visualize the anatomical structure located in the same area as the shear wave's propagation,



the tube was used as a reference in order to localize its indentation observed on the MRI anatomical image (Fig 3). Once the tube was attached around the subject's thigh with the same previous criteria, the ultrasound transducer (13 MHz) was placed perpendicularly and moved around it until to visualize both vastus medialis (VM) and Sartorius (Sr) muscles as represented on the sagittal MRI anatomical image. Then, marks were done with a pen on the skin to identify the placement of the probe. Longitudinal ultrasonic images (resolution 960 x 720mm) showing the parallel-oriented pattern of fascicles were recorded in relaxed and contracted (10% and 20% MVC) conditions for each subject using the same visual feedback as for MRE test. Repeatability of ultrasonic acquisitions was done on each subject, the same day but at different times when the muscle was relaxed and contracted. Ultrasound fascicle angles ( $\alpha_{US}$ ) defined as the angle between the subcutaneous adipose tissue-muscle interface and the path of the fascicle (Fig. 2b) were manually measured for each subject at relaxed and contracted states (10% and 20 % of MVC) using a protractor with an accuracy of 0.5°. The reproducibility was done by two different operators indicating a variation of about 0.3°.

### **Statistical analysis**

Paired student's t-tests were performed with the software Statgraphics 5.0 (Sigma Plus, Maryland, USA) to compare the shear wave ( $\alpha_{MRE}$ ) with the fascicle ( $\alpha_{US}$ ) angles, and the shear modulus between the muscle and the subcutaneous adipose tissue. In addition, unpaired student's t-tests were used to analyze the fascicle angle between children and adults at different states. The level of significance was set at  $p < 0.05$ .

## RESULTS

### Comparison between the shear wave ( $\alpha_{\text{MRE}}$ ) and the fascicle ( $\alpha_{\text{US}}$ ) angles

The children showed a shear wave and fascicle angles ( $\alpha_{\text{MRE}}$  and  $\alpha_{\text{US}}$ ) close to the zero value at rest (Fig. 4a, Fig. 4b), representing a muscle structure composed of parallel fascicles. At 10% MVC, shear wave angles significantly ( $p < 0.05$ ) increase ( $\alpha_{\text{MRE}} = 11.6 \pm 3.14^\circ$ , Fig. 4c) due to a parallel-oriented pattern of fascicles visualized through ultrasound acquisitions revealing a similar increase of fascicle angle ( $\alpha_{\text{US}} = 10.6 \pm 2.27^\circ$ , Fig. 4d). At 20% MVC, the shear ( $13.2 \pm 1.77^\circ$ , Fig. 4e) and fascicle ( $10.2 \pm 2.29^\circ$ , Fig. 4f) angles reached a plateau. In addition, it has been found that the children fascicle angle measured in contraction can sometimes be very low (about  $4^\circ$ ) or very high (about  $18^\circ$ ). Indeed, this result is linked to the state of the fascicles at rest, which can be more distended or already in tension compared to the representative fascicle shape observed in muscle's children.

The adults had a propagation of shear waves ( $\alpha_{\text{MRE}} = 13.7 \pm 1.5^\circ$ ) as well as a pattern of fascicles ( $\alpha_{\text{US}} = 14 \pm 0.98^\circ$ ) already oriented with a same angle in a relaxed condition. Then, both angles slightly increase, in the same range, at 10% MVC ( $\alpha_{\text{MRE}} = 16.8 \pm 2.6^\circ$  and  $\alpha_{\text{US}} = 15.4 \pm 2.54^\circ$ ) and at 20% MVC ( $\alpha_{\text{MRE}} = 16.2 \pm 2.29^\circ$  and  $\alpha_{\text{US}} = 17.2 \pm 2.44^\circ$ ).

Similar results between the direction of the wave's propagation ( $\alpha_{\text{MRE}}$ ) and the path of the fascicles ( $\alpha_{\text{US}}$ ) for each muscle condition (relaxed and contracted) were found as well for children as for adults. These results suggest that the propagation of the shear waves follows the path of the fascicles reflecting the muscle architecture.

## Effect of age on the muscle architecture

Figure 5 represents a comparison of the fascicle angle ( $\alpha_{US}$ ) between children and adults when the muscle was relaxed and contracted (10% and 20% MVC).

At rest, the fascicle angle ( $\alpha_{US}$ ) is significantly ( $p < 0.05$ ) different between children and adults indicating changes of muscle architecture from childhood to adulthood. In addition, ultrasound images performed on children's muscle revealed the presence of more connective tissue (perymesium, Fig. 4b), represented by a hyper-echogeneity of the signal and smaller fascicles compared to adults.

At 10% and 20 % of MVC, the fascicle angle is significantly ( $p < 0.05$ ) higher for adults ( $\alpha_{US\_10\% \text{ MVC}} = 15.4 \pm 2.14^\circ$  and  $\alpha_{US\_20\% \text{ MVC}} = 16.2 \pm 2.29^\circ$ ) compared to the children ( $\alpha_{US\_10\% \text{ MVC}} = 10.6 \pm 2.27^\circ$  and  $\alpha_{US\_20\% \text{ MVC}} = 10.2 \pm 2.45^\circ$ ). This result suggests that a small level of contraction induced important changes of muscle architecture for children while only a slight increase of the fascicle angle was found for adults.

## Variation of the shear modulus inside the muscle

The measurements of the shear modulus ( $\mu$ ) within the adults VM muscle in a relaxed state revealed a variation of 1kPa along the muscle thickness ( $\mu_{\text{Adults\_P1}} = 2.87 \pm 0.17 \text{ kPa}$  and  $\mu_{\text{Adults\_P2}} = 3.92 \pm 0.32 \text{ kPa}$ ) (Fig. 1a). Moreover, the phase image showed the propagation of two distinct shear waves travelling separately in the upper and lower parts of the muscle, leading to different stiffnesses.

However, the propagation of the shear waves within the children's muscle showed a unique wave (Fig. 1b) traveling through the VM muscle providing a similar stiffness at different localizations along the muscle thickness.

### **Comparison of stiffnesses between VM and subcutaneous adipose tissue**

Adults shear modulus measured in a relaxed condition within the VM muscle (along the profile #1, Fig. 1a) and within the subcutaneous adipose tissue (along the profile #3, Fig. 1a) showed a lower significant ( $p < 0.05$ ) stiffness ( $1.85 \pm 0.17$  kPa) for the subcutaneous adipose tissue compared to muscle tissue ( $3.08 \pm 0.21$  kPa). This result is not correlated to the Body Mass Index (BMI) measured for each subject.

## DISCUSSION

The originality of this present study was to demonstrate that the magnetic resonance elastography, beyond the measured mechanical parameter (shear modulus), is also able to provide architectural muscle parameter (fascicle angle) and to detect different physiological composition (adipose vs muscle tissues).

In order to show that the direction of the shear wave propagation followed the orientation of muscle fascicule, different experiments were performed using bovine gel phantom, representing muscle taut bands, (Chen et al. 2007) and in vitro bovine muscle specimens (Dresner et al. 2001). Both studies have demonstrated that the direction of the shear wave propagation was guided by the orientation of the fibers. Moreover, in vivo muscle study have assumed that the propagation of the shear waves was guided by the muscle fascicles (Dresner et al., 2001, Bensamoun et al., 2006) but to our knowledge this statement has never been proven. Thus, one of the purposes of this study was to validate this statement by combining ultrasound and MRE techniques. Indeed, the present study has showed a strong correlation between the orientation of the muscle fascicles (fascicle angle) and the direction of the shear wave propagation (wave angle), revealing the muscle geometry and therefore attesting the previous assumption.

To our knowledge, this study is the first to perform simultaneously MRE experiments and ultrasonographic images on children's muscle providing a characterization of the growing muscle process. Indeed, at rest, waves were propagating parallel to the fascicle children muscle reflecting the on-going muscle structure organization, while adults showed oblique waves corresponding to the presence of already oriented muscle fascicles. In contraction, MRE technique showed the propagation of shear waves in an oblique direction as well for children as adults. Ultrasound images have further characterized this obliquity showing a

higher increase (about  $10^\circ$ ) of the fascicle angle for children compared to adults (about  $3^\circ$ ). The fascicle angles database could be used to develop appropriate models for mimicking children and adults muscle geometry behavior so as to better predict the muscle mechanical properties.

The association of the phase and ultrasound images recording with magnetic resonance elastography and ultrasound techniques, respectively, has demonstrated that the shear modulus is correlated to the muscle architecture. Indeed, we have previously characterized the VM stiffnesses of young adults (20-30 years) (Debernard et al, 2010) and a gradually increase (about 2-3kPa) of the shear modulus was measured from the rest to the contracted (10% and 20% MVC) conditions due to tenser fascicles, and not to a change in fiber orientation which stays approximately the same (variation of 1 degree) at 10% and 20% MVC. We also have previously demonstrated (Debernard et al., 2010) that at 10% MVC children have already reached their maximal stiffness, which may be explained by the high increase of fascicle angle (about  $10^\circ$ ) demonstrating the on-going structural organization of the muscle, leading to a random and uncontrolled fibers recruitment.

Shear modulus measured at different locations within the VM muscle revealed that adult muscle tissue is composed of a less homogeneous media compared to children's due to the ongoing growing process. Stiffness variation found inside the same adults' muscle may be due to differences in fiber tension, which seems to be tenser close to the aponeurosis. To our knowledge, these changes of stiffness inside the muscle have never been characterized and this information is of great importance for the follow-up of patients. Therefore, the mechanical properties must be measured in the same region inside the muscle tissue.

In addition to the stiffness changes, the composition of the media can also alter the wave length and therefore the shear modulus. Thus, stiffness of subcutaneous adipose muscle tissue was found significantly lower than the one measured in the muscle. This information is

capital for future MRE experiments performed on patients with myopathy such as Dystrophy Muscular Duchennes, since muscle tissue is progressively replaced by adipose tissue (Schiaffino and Hanzlikova, 1970) leading to an alteration of the muscle fascicles orientation and as a consequence to different directions of waves fronts. Thus, MRE technique could be a potential clinical tool to characterize the mechanical properties of the muscle in patients suffering from neuromuscular disorders, particularly during therapeutic trials.

## **AKNOWLEDGEMENT**

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## **CONFLICT OF INTEREST**

All authors have no conflict of interest to disclose.

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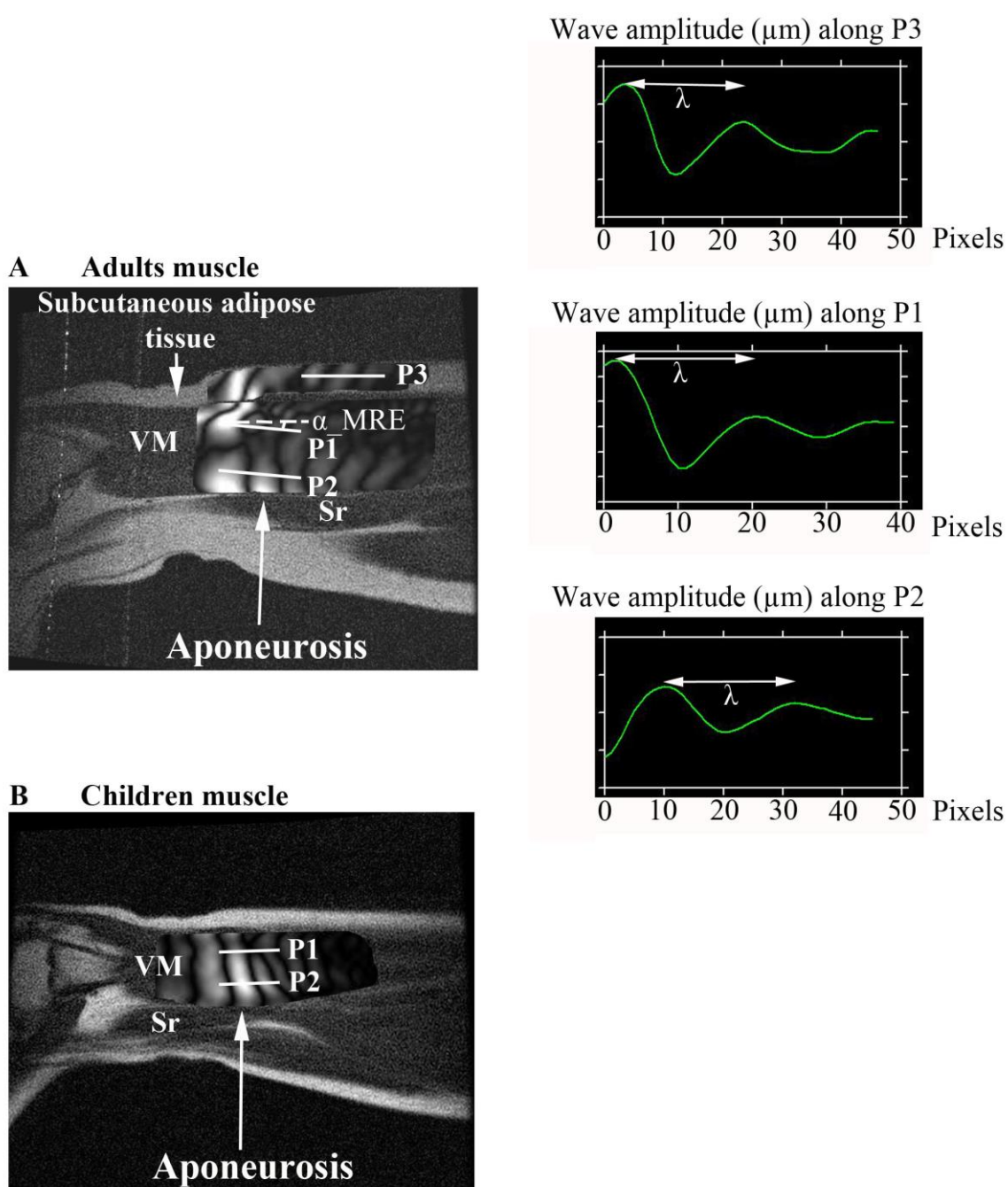
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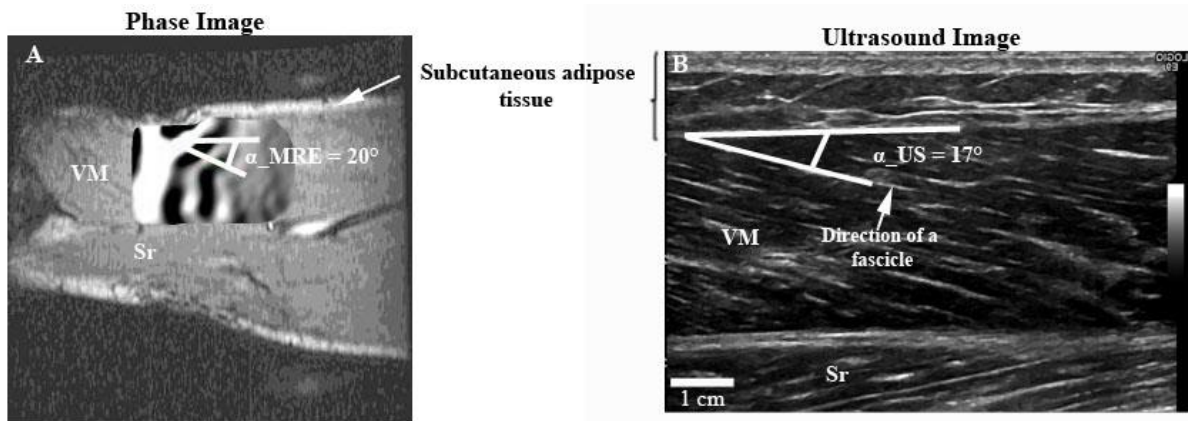
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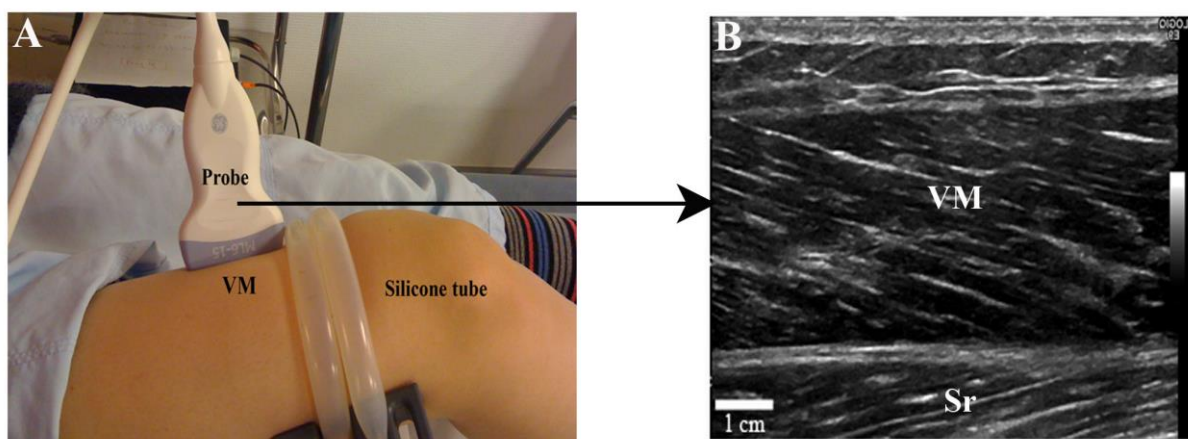
## Figure Legends



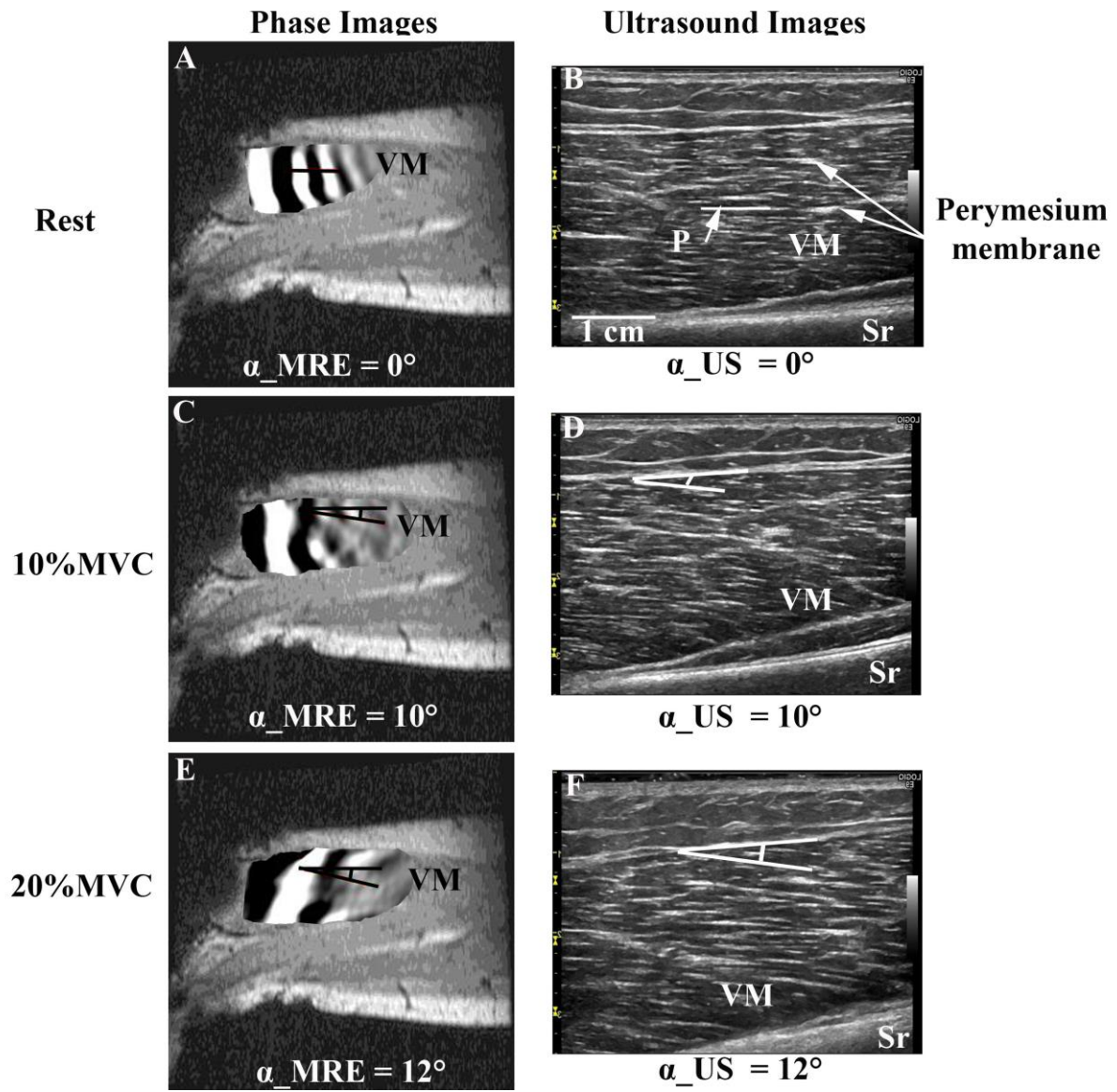
**Figure 1:** Propagation of shear waves inside the relaxed vastus medialis (VM) of adults (A) and children (B). Three profiles P1, P2, P3 were placed in the upper part of VM, close to the aponeurosis membrane and inside the subcutaneous adipose tissue, respectively. The wave displacement amplitude was illustrated for each of the three profiles placed in the adult muscle.



**Figure 2:** Phase image (A) with its corresponding ultrasound (B) image recorded in the same sagittal planes of relaxed adult vastus medialis (VM) and Sartorius (Sr) muscles. A: Representation of the shear wave angle ( $\alpha_{MRE}$ ) measured between the subcutaneous adipose tissue-muscle interface and the direction of the shear wave propagation. B: Representation of the fascicle angles ( $\alpha_{US}$ ) measured between the subcutaneous adipose tissue-muscle interface and the path of a fascicle.

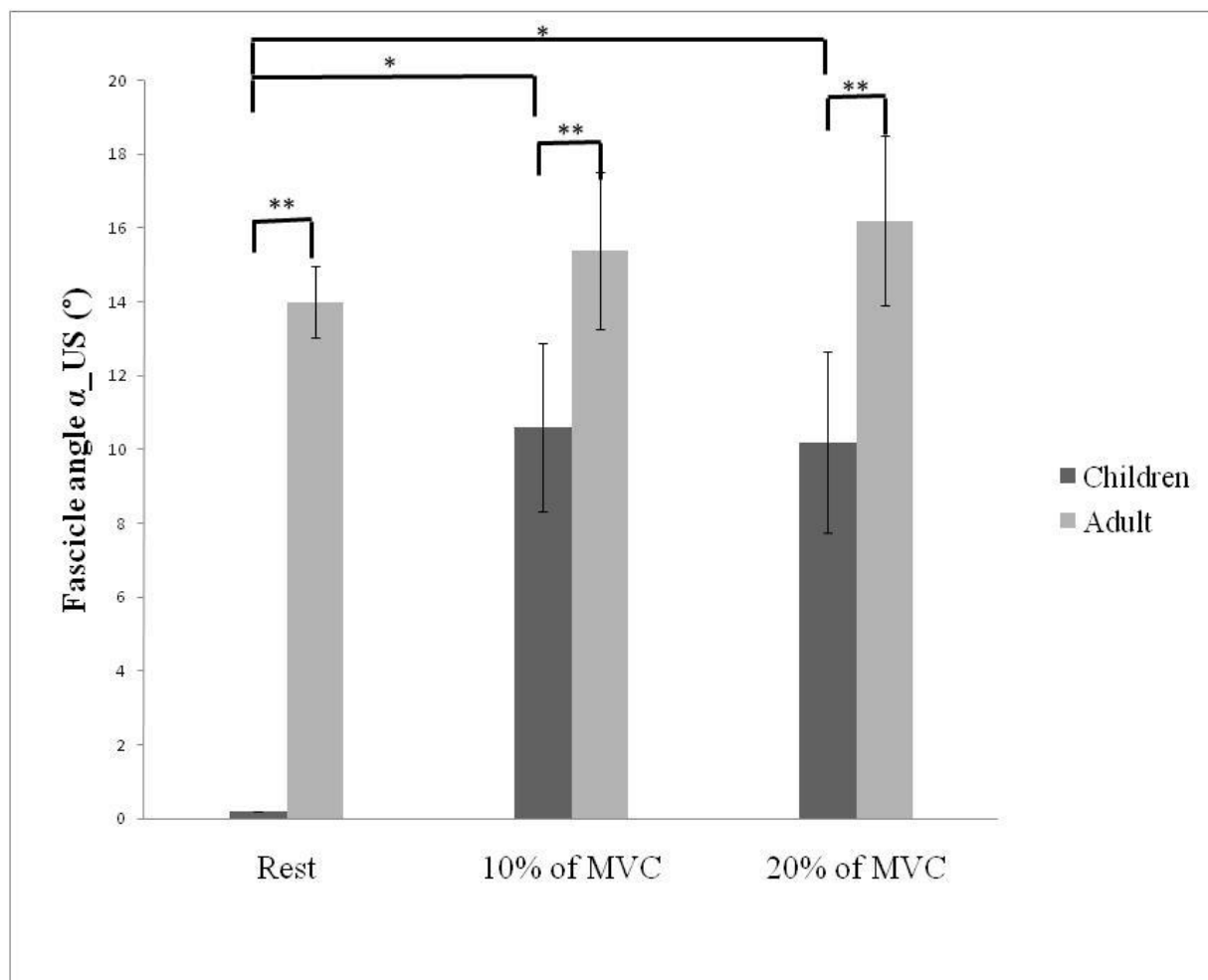


**Figure 3:** A. Placement of the ultrasound probe on the vastus medialis (VM) muscle in order to visualize the VM muscle architecture (B).



**Figure 4:** Phase images and its corresponding ultrasound images for children at different muscle conditions (rest, 10% and 20% MVC).





**Figure 5:** Representation of the VM fascicle angle  $\alpha_{US}$  in relaxed and contracted states (10% MVC and 20% MVC) for children and adults. ( \*\*  $p < 0.05$  and \*  $p < 0.1$ ).