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In vivo characterization of the muscle viscoelasticity in passive and active conditions using multifrequency MR elastography (MMRE)

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ABSTRACT

This study aims to develop a viscoelastic database for muscles (VM: vastus medialis and Sr: sartorius) and subcutaneous adipose tissue with multifrequency magnetic resonance elastography (MMRE) coupled with rheological models. MMRE was performed on 13 subjects, at 70-90-110Hz, to experimentally assess the elastic properties (μ) of passive and active (20% MVC) muscles. Then, numerical shear modulus (μ) and viscosity (η) were calculated using three rheological models (Voigt, Zener, springpot). The elastic properties, obtained with the springpot model, were closer to the experimental data for the different physiological tissues ($\mu_{Springpot_VM_Passive}$= 3.67 ± 0.71 kPa, $\mu_{Springpot_Sr}$= 6.89 ± 1.27 kPa, $\mu_{Springpot_Adipose\ Tissue}$ = 1.61 ± 0.37 kPa) and at different muscle states ($\mu_{Springpot_VM_20\%MVC}$ = 11.29 ± 1.04 kPa). The viscosity parameter increased with the level of contraction ($\eta_{_VM_Passive_Springpot}$ = 4.5 ± 1.64 Pa.s vs $\eta_{_VM_20\%MVC_Springpot}$ = 12.14 ± 1.47 Pa.s) and different muscles ($\eta_{_VM_Passive_Springpot}$ = 4.5 ± 1.64 Pa.s vs $\eta_{_Sr_Springpot}$ = 6.63 ± 1.27 Pa.s). Similar viscosities were calculated for all tissues and rheological models. These first physiologically realistic viscoelastic parameters could be used by the physicians to better identify and monitor the effects of muscle disorder, and as a database for musculoskeletal model.

Key words: muscle; viscosity; elasticity; rheological models; magnetic resonance elastography.

1. INTRODUCTION

Muscle is a complex structure whose primary function is to produce a voluntary contraction in order to generate locomotion and movement. A global analysis of the muscle condition can be performed by the study of functional properties including muscle excitability, contractility, extensibility and elasticity. Nevertheless, there is a need to characterize other muscle characteristics that are more related to muscle intrinsic components, such as viscosity. As a consequence, a viscoelasticity database will be of interest in clinical practice, to have a complete characterization of the muscle tissue and to better define the underlying pathophysiology of some muscle diseases that may account for functional changes.

The elastic properties of the muscle were recently analyzed with novel non-invasive elastography methods, including ultrasound (US) and magnetic resonance (MR) imaging, which are determined by the analysis of the shear wave's displacement. Among ultrasound methods, the sonoelastography and transient elastography techniques, using a vibratory device associated to an US probe, measured the Young modulus of the quadriceps muscle[22] as well as the hardness of the biceps brachii[13] during isometric contractions. In addition, real-time sonoelastography was used to investigate the elastic properties of pathological thigh muscles (Bethlem myopathy) showing stiffer areas of abnormal muscle than the normal-appearing areas[12]. In parallel, MR elastography technique, composed of pneumatic drivers, generating shear waves, analyzed the wave attenuation coefficient (α) as a parameter of the muscle quality[10] and the shear modulus (μ) [3,8,11,23,24] as a study of the muscle elastic properties. Thus, an elastic database on healthy thigh muscles[4,8] and calf muscles[24] was established, and the elastic parameters were compared to those recorded for muscles affected by myositis[22] or

hyperthyroidy[4], showing the feasibility of the MR elastography technique to differentiate healthy muscle tissues from pathological ones.

While functional properties were mainly quantified by the elastic component of the tissue, the viscosity parameter (η) was also investigated to characterize the micro structural changes. Thus, US and MR elastography techniques were further developed with new experimental protocols, such as multifrequency tests and advanced inversion algorithms[25, 26]. Recent algorithms provide a complex shear modulus whose imaginary part corresponds to the loss modulus, and consequently the viscosity. Thus, viscosity databases were performed on healthy brain by Sack et al. (2008), at different ages for men and women[26] and also on the cerebellum[28]. Klatt et al. (2007) demonstrated that human brain had a higher viscosity than liver tissue, which itself presents a higher viscosity than fibrotic livers[1,20]. Concerning skeletal muscle tissue, ultrasound techniques (sonoelastography) were the first method used to determine the muscle viscosity, and Hoyt et al. (2008) showed an increase of the muscle functional parameters (elasticity and viscosity) in contracted state[17]. Recently, a novel supersonic shear imaging technique has succeeded to measure the brachialis muscle viscosity along and perpendicularly to the muscle fibers[18].

Viscoelasticity (μ, η) parameters were determined with various rheological models (Voigt, Zener, Maxwell, Springpot), composed of spring and dashpot[27]. In the literature, the main rheological models, employed to assess the viscoelastic (μ, η) characteristics of the biological soft tissues, is the Voigt model, due to its simple composition[6,7,14,17]. To our knowledge, the muscle viscosity was determined by one study[20], using multifrequency MR elastography (MMRE) test with a rheological model (Springpot). However, springpot model being composed of three independent constitutive parameters (the elasticity μ, the viscosity η and a powerlaw behavior parameter α related to the excitation pulsation: ω^{α}), the viscosity

parameter was fixed (η = 1 Pa.s or η = 10Pa.s) in order to assess the passive and active muscle elasticity through the variation of the coefficient α[20,21].

Thus, this study aims to assess elasticity (μ) and viscosity (η) parameters of individual thigh muscles (vastus medialis and sartorius muscles), in relaxed and contracted states, using multifrequency MR elastography (MMRE) tests and rheological models (Voigt, Zener and Springpot). In addition to the muscle tissue, the viscoelastic (μ, η) properties of the subcutaneous adipose tissue will be characterized, due to fatty infiltration happening in myopathy and ageing process.

2. METHODS

2.1 Participants

Thirteen healthy subjects (11 males and 2 females, average age = 36.6 ± 4.6 yrs, ranging from 23 to 60 years old, mean Body Mass Index: BMI = 24.2 ± 1.5 kg/m²) were tested using multifrequency magnetic resonance elastography (MMRE) tests. These subjects were free from muscle degeneration and did not present history of muscle pathology. This study was approved by the ethical committee (Comite de Protection des Personnes Nord-Ouest II, Amiens), and all the volunteers have signed consent forms.

2.2 Multifrequency Magnetic Resonance Elastography (MMRE) tests on thigh muscles and subcutaneous adipose tissues

MMRE tests allowed for an experimental quantification of the elastic properties (μ) and for a qualitative observation of the viscous (η) component for the vastus medialis and sartorius muscles, in active and passive conditions, as well as for the subcutaneous adipose tissue.

Experimental setup

The experimental protocol has been already published [8, 9], and is briefly summarize here. All MR scans were acquired in a single session on a 1.5T General Electric HDx scanner using a surface coil, placed around the subject's thigh. The volunteer lied down on an adult leg press[3], with the knee flexed to 30° and the right foot placed on a footplate connected to a load cell (SCAIME, Annemasse, France) to record the muscular force. A visual feedback (LABVIEW program) of the developed muscle force was back-projected to the MR room allowing the patients to control their developed force during MRE acquisitions. A thin tube, wrapped around the subject's thigh, was connected to a pressure driver to generate shear waves at

different frequencies (70Hz, 90Hz and 110Hz) enabling the quantification of the viscoelastic (μ, η) properties of the vastus medialis (VM) and sartorius (Sr) muscles, in passive and active (20% of the maximum voluntary contraction: MVC) states. The sartorius muscle was only analyzed in a relaxed condition due to the isometric test. The three frequencies (70Hz, 90Hz and 110Hz) were chosen to be close to the reference frequency (90Hz) obtained with the present tube driver, mainly used for muscle elasticity characterization[3,8,10,23,24]. Throughout the MMRE muscle test, the shear waves were also propagating inside the subcutaneous adipose tissue, allowing also for an analysis of the viscoelastic properties in that physiological tissue.

MMRE images were acquired 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 applied for each magnetic resonance elastography tests at each frequency. The scan time at 70Hz, 90Hz and 110Hz was 38 s (TR/TE of 54 ms/24.6 ms), 32 s (TR/TE of 56 ms/23.2 ms) and 33 s (TR/TE of 50 ms/32.1 ms), respectively.

Image processing and data analysis

MMRE test acquires phase images, showing the displacement of the shear wave within tissues under investigation *(*vastus medialis, sartorius and the subcutaneous adipose tissue, Fig. 1A) at the three different frequencies (Fig.1B,1C,1D). Then, the same operator manually prescribed a profile (P) with an accuracy ±5°, in the direction of the shear wave propagation, following the muscle fascicule paths[9], inside the vastus medialis (P1, Fig.1B), the sartorius (P2, Fig.1B) and also within the subcutaneous adipose tissue (P3, Fig.1B) where the shear waves were travelling in the longitudinal direction. It can be noted that for each investigated frequency, different profiles (P1, P2, P3) were plotted in the same tissue area.

The wavelength (λ) was computed from the phase images between two consecutive peaks and averaged from the four offsets. The shear modulus was measured in the elastic case from Helmholtz equation ($\mu = \rho\lambda^2 f^2$, with the density ρ = 1000 kg/m^3 for the muscle and the subcutaneous adipose tissue) at each frequency (μ_{70Hz}, μ_{90Hz}, μ_{110Hz}), assuming that the biological tissues (muscles and subcutaneous adipose tissue) were linearly elastic, locally homogeneous, isotropic and incompressible. Subsequently, the experimental velocities ($V_{experimental} = \lambda f$), calculated for each frequency, were replaced in each rheological model in order to measure the viscoelastic parameters.

2.3 Determination of in vivo elasticity (μ) and viscosity (η) parameters using rheological models

The viscoelastic properties were characterized with three different rheological models (Voigt, Zener and springpot). The rheological models provide a numeric quantification of a complex shear modulus (G*, kPa) composed of elastic (μ, kPa) elements and viscous (η, Pa.s) components as a function of the complexity of the model. The mechanical illustration of the elastic behavior is always represented by a spring element showing the global elasticity of the muscle structure. For the viscous component the mechanical illustration is a dashpot element representing the intrinsic structure of the muscle (for instance the friction between muscle fibers). Voigt model (Fig.2A) was chosen for its simple composition (only two-elements: one spring and one dashpot). Zener model (Fig.2B) was chosen for its similarity to Hill's model[16] representing the parallel elastic component (μ_1) and the series elastic component (μ_2) of the muscle structure. A fractional model, springpot (Fig.2C), was also chosen for its viscoelastic behavior which is dependant to a weighting factor. An identification method, based on mean squared analysis and implemented in Matlab R2008b software (The Matworks, Inc., Natick, MA) was used to assess the rheological coefficients (μ, η). The objectif of this Matlab

program was to minimize a cost function between the experimental velocities ($V_{experimental}$ = λf) from the multifrequency MRE tests and theoretical velocities (V_{G^*}) from Helmholtz equation[5], in a viscoelastic case, for each rheological model (figure 2) :

$$V_{G^*}{}^2 = \frac{2 \cdot |G^*|^2}{\rho \cdot (|G^*| + \mathrm{Re}(G^*))}$$

2.4 Statistical analysis

Differences were assessed with the software Statgraphics 5.0 (Sigma Plus, Maryland, USA), using one way factor ANOVA and paired *t*-test with a view 1) to compare the experimental and numerical elastic components at 90Hz (the reference frequency) in order to determine which rheological models best fit the experimental data and 2) to compare the viscous component between the three rheological models. This analysis was realized for each muscle type (VM, Sr), muscle state and subcutaneous adipose tissue. The significance was fixed to $P < 0.05$.

3. RESULTS

Table 1 illustrates the viscoelastic (μ and η) properties of the different biological tissues measured experimentally (MMRE tests) and numerically (rheological models).

3.1 Comparison of experimental and numerical elastic properties

Experimental MMRE results are in accordance with previous magnetic resonance elastography studies[3,8,24], and showed an increase of the VM elastic properties from the passive to the active states, whatever the frequencies are. In addition, the elastic properties of the passive sartorius were also found higher than the ones of the vastus medialis at rest[3]. At last, the subcutaneous adipose tissue exhibits a lower elastic property compared to the present muscle tissues[9]. Reproducibility of the shear modulus, as computed as the standard deviation of three successive MRE tests[9], at 70Hz, 90Hz and 110Hz was 0.13 kPa, 0.21kPa, 0.31kPa at rest and 0.47kPa, 0.75kPa, 1.04kPa in contracted, respectively. Knowing that the frequency 90Hz is considered as the reference frequency, with the present driver, for the magnetic resonance elastography muscle characterization[3,8,9,10], the experimental shear modulus measured at this frequency (μ_{90Hz}) was used to be compared with those generated by each rheological model (Voigt, Zener and Springpot) (Table 1). The comparison of the experimental (μ_{90Hz}) and numerical shear modulus reveals similar elastic properties with the springpot model for the vastus medialis in passive ($\mu_{90Hz\ _VM_Passive}$= 3.90 ± 0.26 kPa vs. $\mu_{Springpot_VM_Passive}$= 3.67 ± 0.71 kPa, Figure 3A) and active ($\mu_{90Hz_VM_20\%MVC}$ = 11.03 ± 1.21 kPa vs. $\mu_{Springpot_VM_20\%MVC}$ = 11.29 ± 1.04 kPa, Figure 3B) conditions, for the sartorius muscle ($\mu_{90Hz\ _Sr}$= 6.76 ± 0.44 kPa vs. $\mu_{Springpot_Sr}$= 6.89 ± 1.27 kPa, Figure 3C), and also for the subcutaneous adipose tissue ($\mu_{90Hz\ _Adipose\ Tissue}$= 2.0 ± 0.33 kPa vs. $\mu_{Springpot_\ Adipose\ Tissue}$ = 1.61 ± 0.37 kPa, Figure 3D) (Table 1). The present results demonstrated that the elastic properties obtained with the springpot model for different type of tissues (vastus medialis, sartorius and

subcutaneous adipose tissue) at different states (passive vs. active) were close to the experimental data. The results showed that the two other models (Voigt and Zener) were also adapted to predict the elastic properties. It must be noted that Voigt model provides lower elastic properties compared to those experimentally (MMRE tests) measured for the passive and active vastus medialis, the subcutaneous adipose tissue and a slight difference with the sartorius. In the same way, the first Zener's elastic component (μ_1) showed also lower muscle elastic properties than MMRE results for the passive and active vastus medialis, with lower elasticity (μ_1) compared to Voigt model (Table 1). The second Zener's elastic components (μ_2) varies as a function of the muscle types (VM vs. Sr, Figure 3A-3C), and as a function of the muscle states (passive vs active, Table 1). Indeed, the μ_2 value corresponding to the passive (Figure 3A) and active (Figure 3B) vastus medialis, was similar as the results obtained with springpot model and was in the same range as those experimentally (MMRE) measured (Table 1). In contrary, the second Zener's elastic components for the sartorius muscle (Figure 3C, Table 1) exhibited different range of μ_2 than those obtained experimentally (MMRE) and also numerically (Springpot). In addition, it can be noticed that the second Zener's elastic components (μ_2) is always higher than the first one (μ_1) (Table 1).

3.2 Quantification of the viscosity (η) with rheological models

The frequency-dependant shear modulus measures for the different tissues under investigation, demonstrated a qualitative viscous (η) behavior which was quantified using rheological models.

The comparison of the quantitative viscosity calculated for each biological tissue with the three rheological models showed the same range of values depending on the rheological models (Table 1). It can be noticed a slight increase of the viscosity value with the Springpot

model compared to the data provided by Zener and Voigt models, for all the physiological tissues under investigation.

Following results on the viscosity was derived from springpot model. For the vastus medialis muscle, the viscosity parameter increased with the level of contraction ($\eta_{_VM_Passive_Springpot}$ = 4.5 ± 1.64 Pa.s vs $\eta_{_VM_20\%MVC_Springpot}$ = 12.14 ± 1.47 Pa.s). The sartorius muscle revealed a higher viscosity than the passive vastus medialis muscle ($\eta_{_VM_Passive_Springpot}$ = 4.5 ± 1.64 Pa.s vs $\eta_{_Sr_Springpot}$ = 6.63 ± 1.27 Pa.s). The subcutaneous adipose tissue exhibited lower viscosity ($\eta_{_Adipose\ Tssue_Springpot}$ = 1.93 ± 0.32 Pa.s) compared to the present muscle tissues (vastus medialis and sartorius).

4. DISCUSSION

The relevance of this study was to characterize the mechanical properties of different physiological tissues (muscle and subcutaneous adipose tissue) with the set up of a viscoelasticity (μ, η) database, using Multifrequency Magnetic Resonance Elastography (MMRE) tests and rheological models. This novel database could be of use for physicians to better elucidate the pathophysiology of a muscle disorder and to better monitor effects of muscle disease. Moreover, the characterization of the collageneous tissue is of importance in myopathy and ageing process, due to its infiltration inside the muscle.

The experimental elastic properties (μ), established for the present soft tissues under investigation, (vastus medialis, sartorius and subcutaneous adipose tissue), are in agreement with previous publications, demonstrating changes of the shear modulus according to the muscle type[2,15,18], muscle condition[3,8] and physiological media[9]. These results attest the suitability of the applied MMRE tests to determine the soft tissues elastic characteristics.

Present study's novelty is to quantitatively determine the viscosities of the passive and active muscle as well as those of the subcutaneous adipose tissue using MMRE and rheological models. Indeed, a previous MRE study of the skeletal muscle was performed by Klatt et al. (2010), with the springpot model, where the viscosity parameter was fixed compared to the present study which quantify this parameter. Besides, the assessment of the viscoelastic parameters was performed on a group of muscles (femoral muscles), while this present study clearly establishes changes of viscosity values depending on the muscle type (vastus medialis vs. sartorius) and the muscle state (passive vs active). The viscous behavior can be interpreted in terms of the friction's force, acting between the muscle fibers. From this interpretation, it is expected an increase of the viscosity with the level of contraction as it was found for the vastus medialis from the passive to the active state. Additionally, this last result is in accordance with the literature[14]. Differences in muscle viscosity in the literature can be

due to the elastography techniques (sonoelastography, transient elastography, supersonic shear imaging, magnetic resonance elastography), the choice of the rheological models (Voigt or Springpot) or the muscle under investigation[14,17]. Moreover, the narrow-band frequencies used in the present study could be also a limitation of the identification process and further studies using a wider range of frequencies could be performed.

The present study showed that the three rheological models (Voigt, Zener and springpot) are applied for the assessment of the viscous characteristics for muscles and subcutaneous adipose tissue. In addition to Voigt and springpot models, the introduction of the Zener model gives also insights into muscle physiology using MMRE experiments. Indeed, the first shear modulus (μ_{1_Zener}) may be associated with the elastic component of Hill's model which reflects the connective tissues (fascia, epimysium, perimysium and endomysium) surrounding the muscle structures. Interestingly, the value of the first shear modulus (μ_{1_Zener}), calculated for the passive vastus medialis, was found in the same range as the experimental ones of the subcutaneous adipose tissue. This funding reveals the capability of Zener model to characterize the elastic properties of collageneous structure. Nevertheless, as the second Zener's elastic component (μ_{2_Zener}) was found higher than the other numerical elastic values, it might be concluded that Zener model should not be used to define the muscle elastic properties.

In conclusion, this study has demonstrated that different rheological models could be implemented in clinical MR software to provide clinicians with muscle viscosity. The accuracy of the viscoelastic parameters could be improved with further studies using a wider range of frequency. This study is the first physiologically realistic viscoelastic database, which could be used for musculoskeletal model.

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TABLE

		Vastus Medialis (VM)		Sartorius (Sr)	Subcutaneous Adipose Tissue
		Passive N = 11	Active (20% MVC) N = 11	Passive N = 8	N = 9
MMRE Tests Elastic properties	Wavelentgh (λ, mm)	70 Hz: 25.77 ± 0.70	70 Hz: 43.39 ± 2.40	70 Hz: 30.80 ± 0.64	70 Hz: 18.05 ± 2.03
		90 Hz: 23.91 ± 0.70	90 Hz: 36.18 ± 2.10	90 Hz: 28.21 ± 0.93	90 Hz: 15.37 ± 1.38
		110 Hz: 18.98 ± 0.90	110 Hz: 31.93 ± 2.10	110 Hz: 26.11 ± 1.06	110 Hz: 13.97 ± 1.76
	Shear Modulus (μ, kPa)	70 Hz: 3.28 ± 0.18 (*)	70 Hz: 9.57 ± 0.96 (*)	70 Hz: 4.61 ± 0.19	70 Hz: 1.66 ± 0.41
		90 Hz: 3.90 ± 0.26 (*ΔΘΨΩ)	90 Hz: 11.03 ± 1.21 (*ΨΩ)	90 Hz: 6.76 ± 0.44 (ΔΘ)	90 Hz: 2.00 ± 0.33 (ΘΨ)
		110 Hz: 4.34 ± 1.20 (*)	110 Hz: 12.92 ± 1.65 (*)	110 Hz: 8.13 ± 0.69	110 Hz: 2.50 ± 0.61
Rheological models Viscoelastic properties	Voigt	μ: 2.64 ± 0.20 (ΨΩ)	μ: 7.92 ± 1.60	μ: 4.13 ± 0.69	μ: 1.04 ± 0.75
		η: 3.27 ± 0.38	η: 8.88 ± 1.35	η: 5.38 ± 0.89	η: 1.73 ± 0.46
	Zener	μ_1: 1.79 ± 0.40 (Ω)	μ_1: 7.80 ± 1.77	μ_1: 3.08 ± 0.81	-
		μ_2: 4.46 ± 1.52	μ_2: 13.00 ± 1.39	μ_2: 12.00 ± 2.76	
		η: 3.57 ± 0.92	η: 11.58 ± 2.46	η: 6.37 ± 1.13	
	Springpot	μ: 3.67 ± 0.71	μ: 11.29 ± 1.04	μ: 6.89 ± 1.27	μ: 1.61 ± 0.37
		α: 0.34 ± 0.07	α: 0.68 ± 0.12	α: 0.65 ± 0.09	α: 0.60 ± 0.14
		η: 4.50 ± 1.54 (□ΛΣ)	η: 12.14 ± 1.47 (□Σ)	η: 6.63 ± 1.27 (ΛΣ)	η: 1.93 ± 0.32 (Σ)

TABLE 1. Representation of the experimental MMRE data (wavelength: λ and shear modulus:μ) and the viscoelastic (μ:kPa, η:Pa.s) values obtained from the three rheological models (Voigt, Zener and springpot) for the active (20% MVC) and passive vastus medialis (VM), the passive Sartorius (Sr) and the subcutaneous adipose tissue. The empty box means that no data was calculated for the subcutaneous tissue whom the composition is not related to Hill' model. Values with similar symbols (*ΔΘΨΩ□ΛΣ) showed significant difference ($P<0.05$).

FIGURE LEGENDS

FIGURE 1. Phase image, illustrating the shear wave propagation within the vastus medialis, (VM), sartorius (Sr) and subcutaneaous adipose tissue (A), obtained at 70Hz (B), 90Hz (C) and 110Hz (D). Profiles (P) were plotted along the direction of the shear wave's displacement inside both muscles (#P1: VM, #P2: Sr) and inside the subcutaneous adipose tissue (#P3) to measure the wavelength (λ).

FIGURE 2. Representation of the three solid rheological models: Voigt (A), Zener (B) and Springpot (C). G* represents the shear complex modulus, μ is the shear stiffness (kPa), η is the viscosity (Pa.s), ω is the excitation pulsation (Hz) and α being the powerlaw behavior parameter.

FIGURE 3. Quantification of the viscosity (η) parameter calculated from three various rheological models (Voigt, Zener and Springpot) for the passive (A) and active (B) vastus medialis, the passive Sartorius (C) and the subcutaneous adipose (D) tissue. (* $P < 0.05$)

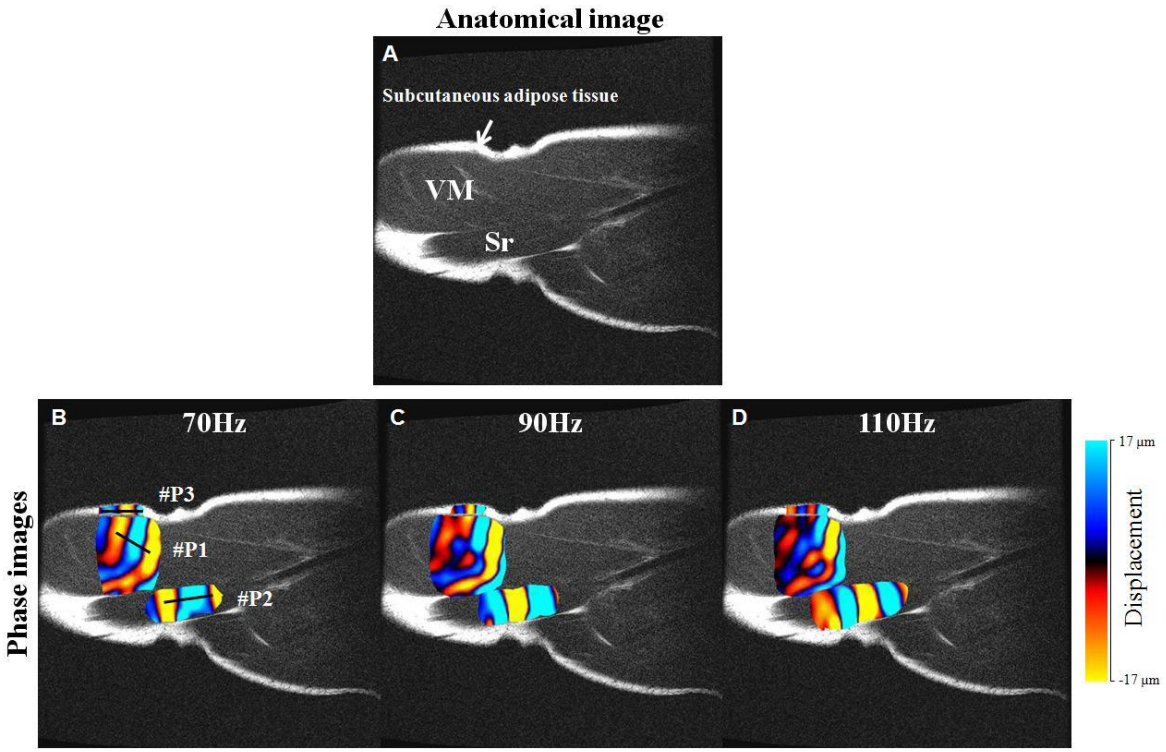


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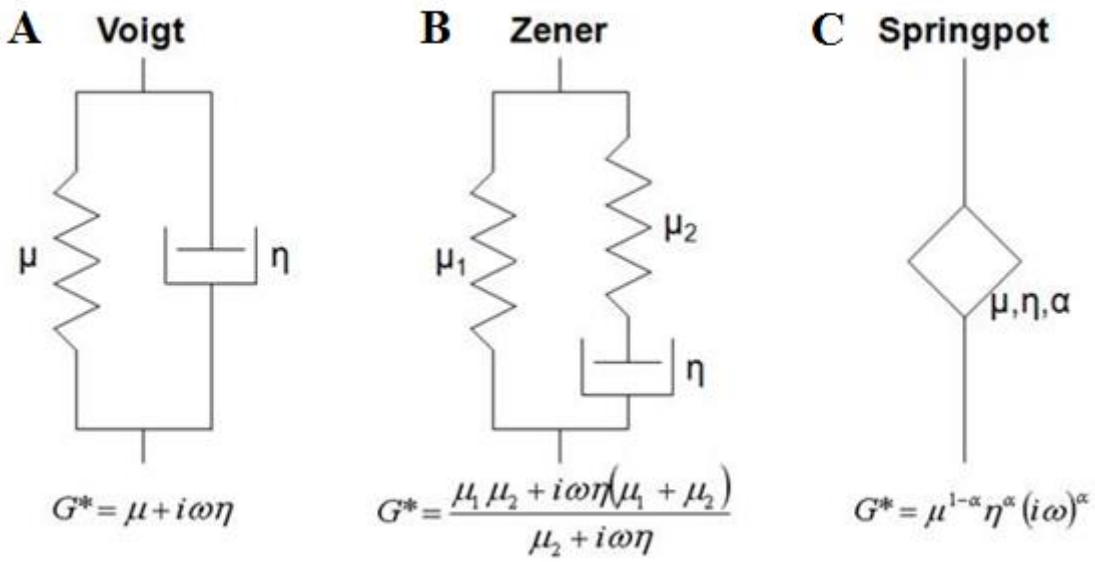


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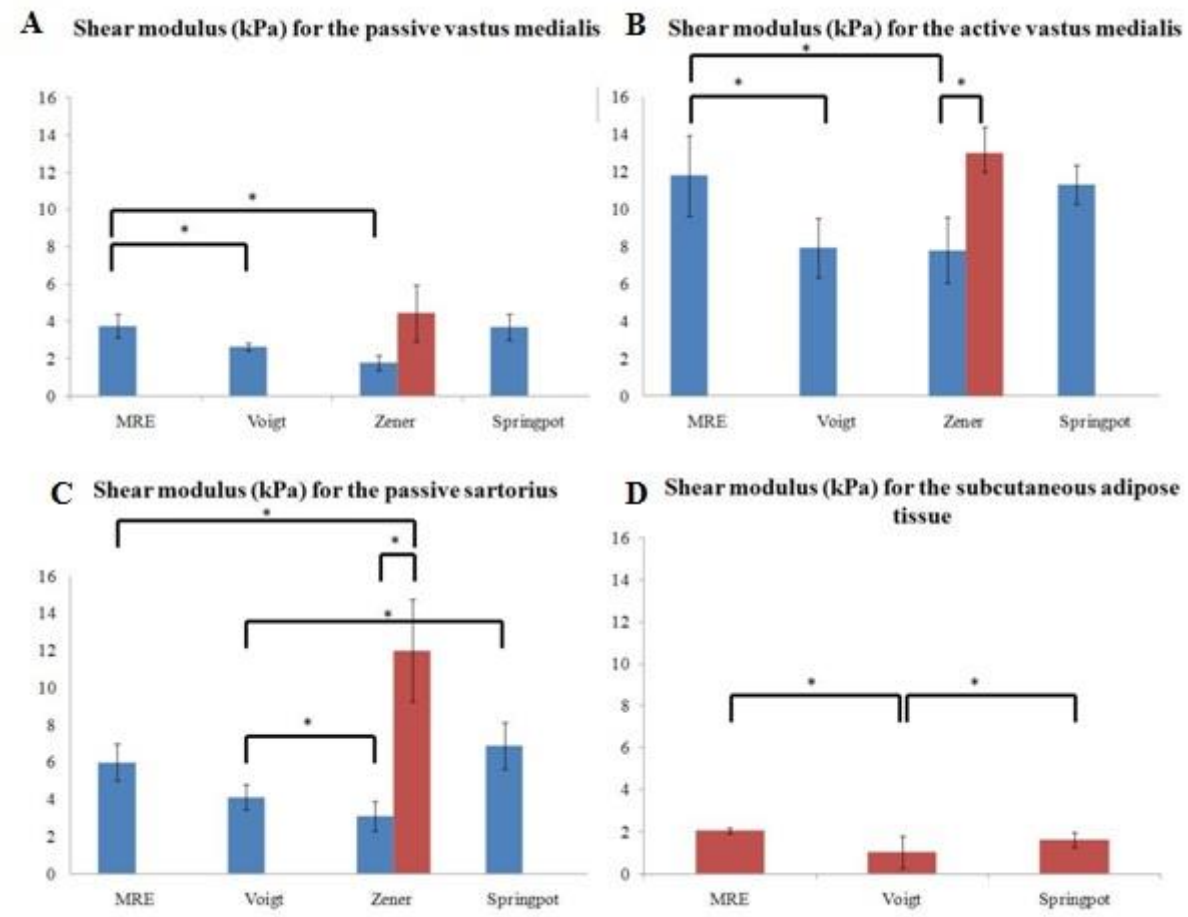


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