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Abstract  Muscle mechanical properties are necessary to improve numerical models of the human body. They have been assessed for animal muscles in studies performed in various experimental conditions. These different experimental protocols may have an influence on muscle response. The aim of this study was thus to evaluate the effect of testing conditions as well as velocity on the passive response of a muscle. Tensile tests at 1 mm.s⁻¹, 10 mm.s⁻¹ and 100 mm.s⁻¹ were performed on a dog muscle in ambient conditions, immersion in 22°C saline solution and immersion in 35°C saline solution. Maximum load F_max and final stiffness K were measured. The influence of velocity and of experimental conditions on these parameters was studied statistically. The parameters were not very sensitive to changes in velocity (increase of 5% for F_max between 1 mm.s⁻¹ and 100 mm.s⁻¹). Nevertheless, they were sensitive to experimental conditions (decrease of 25% for F_max between ambient conditions and immersion in heated saline solution). Consequently, the experimental conditions have an influence on muscle passive response and must be taken into account in the definition of the mechanical properties used in modeling.

Keywords  Experimental conditions, mechanical properties, muscle, tensile test, velocity.

I. INTRODUCTION

Characterization of muscle mechanical properties is necessary to improve numerical models of the human body [1]-[2] or of body parts such as the head and neck [3]-[5] that are used in impact biomechanics. To obtain accurate properties, in vitro experiments have been performed on animal or human muscles. However, these experiments provide data with high variability depending on the muscle tested, the animal species and the experimental protocol used.

For instance, compression tests were performed on samples of porcine muscles at various frequencies (5Hz to 30Hz) in which the sample was placed in a bath of saline solution maintained at 37°C [6]. Compression tests were also performed on porcine samples but at strain rates between 0.005 s⁻¹ and 0.1 s⁻¹ in ambient conditions [7]. Other experiments have been performed at high velocity on bovine [8] and human muscles [9] using specific devices, including experiments using Hopkinson Bars performed on bovine muscle samples in ambient conditions at strain rates up to 2300 s⁻¹ [10]. Regarding the results, high variability is observed. At 30% strain and for a strain rate of 0.1 s⁻¹, a stress of about 3 kPa was measured on porcine muscles in [7] whereas 70 kPa were measured on bovine samples in [8] for the same strain rate and 35% strain. On the other hand, a stress of 20 kPa at 30% strain was measured at 136 s⁻¹ in [9].

There are also in vivo and in vitro experiments performed on entire muscles. They are mainly tensile tests or compression tests [11]-[12] performed at various strain rates ranging, for instance, from 0.0005 s⁻¹ [13] to 25 s⁻¹ [14] and performed on different animals. A few experiments have been conducted on human muscles [15]-[16]. The experimental conditions are numerous. For the in vivo experiments, muscle can be tested in ambient conditions [17]-[19] with a muscle temperature between 22°C and 27°C. Muscle temperature can be controlled by placing the muscle on a heated pad so that it is maintained at 32°C, 35°C or 37°C [11, 20]. The muscle can also be wrapped in gauze soaked with saline solution whose temperature is defined [14, 21]. For the in vitro experiments, muscle can be tested at ambient temperatures and regularly moistened to avoid dehydration [12, 13, 22], or placed in a bath of saline solution whose temperature is controlled [23]-[25]. Variability in the results is also important. For instance, for a dog muscle tested in

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compression and in ambient conditions at 0.016 mm.s⁻¹, a Young’s modulus of 1.71 MPa was identified in [12] whereas it was only 0.047 MPa for live rat muscles compressed at 0.25 mm.s⁻¹ with a temperature of 35°C [11] and 0.447 MPa for a rabbit muscle tested in tension at 0.0005 s⁻¹ [13]. Finally in [21] a decrease between 11% and 15% of the load at failure for a rabbit muscle tested at 25°C and 40°C was observed.

As a consequence, the results obtained from these experiments depend on the muscle tested, on the velocity or strain rate applied to the specimen and on the environmental conditions that are mainly temperature and hydration. The aim of this paper is to focus on the effect of velocity and experimental conditions on the passive response using dog muscles in tension. Experimental conditions are chosen in accordance with those used in the literature. Velocities are chosen on the basis of results obtained from simulations of rear-end car crashes with a validated head and neck model [4, 5].

II. METHODS

Specimens

Twelve extensor carpi ulnaris muscles were removed from six dogs with their bony insertions and aponeurosis. The dogs were of different breed, different gender and had a mean age of 10 years old (min: 8 years old, max: 14 years old). After dissection, each muscle was wrapped in gauze soaked with saline solution and placed in an airtight bag. They were stored at -20°C. The day before the experiment, the muscle was slowly thawed at 4°C for at least twelve hours. The muscle was brought to room temperature two hours before the test.

Experimental Set Up

A specific experimental device was designed to allow tensile tests on the muscle in three different conditions: ambient conditions, immersion in 22°C saline solution and immersion in 35°C saline solution to be close to the animal’s body temperature. The experimental set up (Fig. 1 and Fig.2) allows tensile tests on the muscle that is placed between the jaws. The muscle is fixed in the jaws by cone-point set screws placed on the bony parts of the muscle. Special care is taken to place the muscle in its vertical physiological position. The alignment of the muscle is controlled visually with a plumb line. Once the muscle is maintained in its position, a Plexiglas tank is placed around the muscle. The assembly is then installed on a hydraulic jack [26]. The lower rod that connects the lower jaw is attached to the jack rod to perform tensile tests. The tank may be empty, or filled with cold or heated saline solution by a pump system. The temperature is measured with a thermometer and is maintained by a heating system placed inside the tank.

![Fig. 1. Scheme of the experimental set-up.](image1)

![Fig. 2. Picture of the experimental set-up.](image2)

During the experiment, load is measured with the sensor of 2.5 kN positioned just above the upper jaw (Kistler 9301B, response threshold ≤ 0.02 N, sensitivity ≈ -4 pC.N⁻¹). Displacement is controlled with the hydraulic jack. To obtain another measure of displacement and to monitor the progress of the experiment, a high-speed
camera (Photron APX RS, resolution: 1024x1024) is placed in front of the assembly. The high-speed camera follows targets placed on the lower jaw. The video camera and force sensor are synchronized.

**Experimental Testing**

The experimental protocol started with a stabilization of the muscle mechanical behavior. Fifty loading-unloading cycles were imposed on the muscle (amplitude: 1 mm, frequency: 0.5 Hz, sine-wave signal). This step was performed only once at the beginning of the experiment. The elongations and velocities applied to the muscle were chosen on the basis of simulations performed with a head and neck model [4, 5]. Three rear end collisions [4, 5] (3 g, 11 km.h⁻¹ – 5 g, 16 km.h⁻¹ – 7 g, 25 km.h⁻¹) were simulated. The elongation and elongation rate on the sternocleidomastoideus muscle were measured. The results obtained ranged from 3 to 9% elongation and from 0.1 to 0.7 s⁻¹. Thus for each environment, elongations of 10 mm amplitude at different velocities (1 mm.s⁻¹, 10 mm.s⁻¹ and 100 mm.s⁻¹) were imposed on the muscle to have an average elongation of 5% and elongation rates of 0.005 s⁻¹, 0.05 s⁻¹ and 0.5 s⁻¹. The camera settings were: 50 or 60 fps for 1 mm.s⁻¹ and 500 fps for 10 mm.s⁻¹ and 100 mm.s⁻¹. Loading and unloading were performed at the same velocity. These tensile tests were performed in random order for each experimental configuration. The three environments were tested following this order: ambient conditions, immersion in a saline solution at room temperature (22°C) and finally immersion in a heated saline solution (35°C). An experiment for one muscle lasted 3 to 4 hours.

**Data Analysis**

The load-displacement curves for each muscle, for each velocity and for each experimental condition were plotted. The maximum load $F_{max}$ (N) and the stiffness $K$ (N.mm⁻¹) at the end of the loading phase were studied. The $K$ parameter was calculated using a linear regression on the experimental curve between 7 and 8 mm displacement. To evaluate the influence of velocity and experimental conditions on the passive response of the muscle under tension, a statistical study was performed on the two parameters: the Wilcoxon test for paired signed data was used (5% risk).

**III. RESULTS**

The extensor carpi ulnaris muscles tested had the same shape. They were thin and had a long tendinous part. Muscle total length (including tendons) varied between 160 and 290 mm with a mean value of 207 mm (std: 40 mm) in the initial position under tension. The experimental load-displacement curves all had the same shape as well. As muscle length varies, the maximum elongation was between 3% and 6% for a total stretch of 10 mm. An example of the experimental curves obtained for one muscle for each experimental condition and each velocity is presented in Fig. 3.

![Fig. 3. Experimental load-displacement curves obtained for the left muscle of the 4th dog for each experimental condition and each velocity.](image-url)
Fig. 4. Velocity effect (A) and environment effect (B) on the parameter $F_{\text{max}}$. Mean and standard deviation values. The differences between mean values are given in percentage when $p$-value < 0.05.

Fig. 5. Velocity effect (A) and environment effect (B) on the parameter $K$. Mean and standard deviation values. The differences between mean values are given in percentage when $p$-value < 0.05.
Muscle rupture was never observed during the experiments. The mean values, standard deviations, minimum and maximum values obtained for each parameter (K and F_{\text{max}}) for each velocity and for each configuration are summarized in Table 1.

| TABLE I |
| F_{\text{max}} (N) and K (N.mm\(^{-1}\)): mean value, standard deviation, minimum and maximum values |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Ambient conditions 22°C | Saline solution 22°C | Saline solution 35°C |
|                                 | 1 mm.s\(^{-1}\) | 10 mm.s\(^{-1}\) | 100 mm.s\(^{-1}\) | 1 mm.s\(^{-1}\) | 10 mm.s\(^{-1}\) | 100 mm.s\(^{-1}\) |
| Mean F_{\text{max}}             | 141             | 145             | 149             | 121             | 123             | 124             | 103             | 107             | 108             |
| Std                             | 68              | 70              | 74              | 68              | 67              | 66              | 60              | 61              | 63              |
| Min                             | 69              | 68              | 64              | 49              | 56              | 56              | 39              | 43              | 44              |
| Max                             | 264             | 262             | 269             | 232             | 233             | 228             | 208             | 213             | 215             |
| Mean K                          | 24              | 25              | 25              | 20              | 21              | 21              | 18              | 18              | 19              |
| Std                             | 10              | 11              | 11              | 11              | 10              | 12              | 10              | 10              | 11              |
| Min                             | 13              | 12              | 12              | 8               | 10              | 9               | 7               | 8               | 8               |
| Max                             | 42              | 42              | 42              | 39              | 38              | 45              | 35              | 33              | 37              |

The maximum load was lower when the muscle was immersed in saline solution than when it was tested in ambient conditions. When the muscle was tested in ambient conditions, the maximum load reached was on average 145 N. It was 123 N in cold saline solution and 106 N in heated saline solution. The change in maximum load with velocity was low. For example, under ambient conditions, an increase of 5% between the lowest value (at 1 mm.s\(^{-1}\)) and the highest value (at 100 mm.s\(^{-1}\)) was observed for F_{\text{max}}. The muscle stiffness measured between 7 and 8 mm displacement showed the same trend. The mean value in ambient conditions was 25 N.mm\(^{-1}\). This value decreased to 21 N.mm\(^{-1}\) for a muscle immersed in cold saline solution and was 18 N.mm\(^{-1}\) when the muscle was placed in heated saline solution. The effect of velocity was slightly marked. In cold saline solution, an increase of 5% between the lowest value (at 1 mm.s\(^{-1}\)) and highest value (at 10 mm.s\(^{-1}\)) was noticed for K. Stiffness was calculated using a linear regression whose coefficient of determination was between 0.90 and 0.99.

Statistical analysis showed that the parameters F_{\text{max}} and K were sensitive to the experimental conditions, whatever the change of environment and for each tested velocity (Fig.4, Fig.5). However, K was not sensitive to velocity changes (Fig. 5). F_{\text{max}} was sensitive to changes in velocity between 1 and 10 mm.s\(^{-1}\) and between 1 and 100 mm.s\(^{-1}\), but only when the muscle was immersed in heated saline solution (Fig. 4).

### IV. DISCUSSION

*In vitro* tensile tests were performed on dog muscles. The experimental conditions and velocities imposed on the muscle changed so that their influence on the muscle passive response was assessed.

The extensor carpi ulnaris muscles were frozen at -20°C and slowly thawed before testing. This preservation method may damage muscle mechanical properties [27]-[29]. Nevertheless, in [27], it is shown that a preconditioning implied a reproducible response of the muscle behavior in tension, but that stiffness is decreased compared to fresh specimens. Therefore the experimental results should be interpreted with caution. Besides, muscles were removed from the dogs at a time after death between 12 and 120 hours. This means that some muscles were excised at the end or after rigor mortis. The work presented in [30] highlighted that for ovine muscles tested in compression, the Cauchy stress measured at 10% strain was seven times higher when tested 8 hours after death than 2 hours after death. Hence if fresh tissue had been tested within two
hours after death, the mechanical properties measured in this study would have been probably lower.

Muscles of dogs of several breeds were studied. This diversity may lead to variability in the results. However, it was shown in [31] that the fiber composition of the semitendinosus muscle was the same for different breeds of dogs. The same result was highlighted in [32] for the same muscle and for breeds that, as in the current study, were not selected for racing. As a consequence, this diversity does not affect the results.

However, the muscle volumes and sections vary with the global size of the dog. The load and stiffness values depend on muscle geometry. Mechanical parameters such as the maximum stress would have been a more powerful muscle property to evaluate the experimental and velocity effects, but muscle external geometry was difficult to assess. Experiments on muscle samples with defined geometries are most of the time used to assess such mechanical properties. However, the response obtained is a local one and the tissue is locally damaged which implies also a loss of fluid inside the sample. This can affect and change the tissue response.

In the present study, the passive response of the muscle-tendon complex is evaluated. This global approach could be used in numerical models to define a constitutive law for both the tendon and muscle passive response and avoid continuity problems in the material description. However, to the authors’ knowledge, no evidence was found in the literature that the mechanical properties of animal muscles are the same as human muscles.

During the experiments, a displacement of 10 mm was imposed. This represents an elongation between 3% and 6%, and an average elongation of 5% (standard deviation: 1%). This value is low compared to the experiments reported in the literature. In [17], an elongation of 12% is applied to rabbit muscles. In [13], an elongation of 40% is made on a rabbit muscle. In [33], 20% elongation is reached also on a rabbit muscle. However, in the current study, given the large number of tensile tests imposed on the same muscle (9 in all), a small elongation was preferred to avoid damage of the muscle during the experiment.

Velocities applied to the muscle (1, 10 and 100 mm.s⁻¹) correspond respectively to the following elongation rates: 0.005 s⁻¹, 0.05 s⁻¹ and 0.5 s⁻¹. They are of the order of magnitude of strain rates listed in the literature. In [17], imposed elongation rates are between 0.01 s⁻¹ and 2 s⁻¹. In [13], it is 5.10⁻⁴ s⁻¹. Elongation rates studied in [21] are 0.1 s⁻¹ and 1 s⁻¹ and correspond to velocities of 10 mm.s⁻¹ and 100 mm.s⁻¹. In [34], tensile tests are carried out at the same velocities as in our study (1, 10 and 100 mm.s⁻¹), but elongation rates are not mentioned. Although the elongation rates applied in our study are in the lower range of those presented in the literature, they can highlight their effect on muscle passive response.

The tensile tests are performed in a random order as regards velocities. With this experimental protocol, a steady increase in velocity that could affect the mechanical properties of the muscle is avoided. However, this random order could not be applied for the three experimental conditions, since it was difficult to implement. Indeed, in order to randomly change the experimental conditions, between each step the assembly would have to be completely disassembled and cleaned. This cleaning and reinstallation on the hydraulic jack can take up to an additional hour, and during this period, the muscle would stay at ambient temperature and could deteriorate. To avoid too long a period of experimentation (actual duration: 3 to 4 hours) and possible deterioration of muscle, a constant order of the experimental conditions was chosen.

Fmax is not really sensitive to velocity changes. Only the velocity changes from 1 mm.s⁻¹ to 10 mm.s⁻¹ and from 1 mm.s⁻¹ to 100 mm.s⁻¹ affect Fmax when the muscle is immersed in heated saline solution. However, this influence must be balanced, since an increase in the maximum load of 4% or 5% is noted. This slight effect of velocity may be explained by the experimental protocol in which the initial condition before each tensile test was to recover muscle initial length. Relaxation could affect the reloading curve. This result is not in accordance with the literature. In [14] and [21], a significant effect of velocity on the load at failure of muscle was observed but for larger elongations than those studied here. However, in [33], the effect of velocity was not noticed on the measured load at 12% or 20% elongation for changes in velocity between 0.08 mm.s⁻¹ and 16.6 mm.s⁻¹. They explain that for an elongation below 12%, muscle has an elastic behavior, hence the lack of velocity effect on muscle response. Our results are in accordance with this last study and the maximum elongation applied on the muscle is 6%.

However, in [7] small changes in velocity influence the load response in compression even for small strains. This difference with the literature may be related to the experimental protocol. A lot of tensile tests are performed on the same muscle and this can locally damage some fibers within the muscle and tendon tissue. A plastic strain can appear and is not compensated at the beginning of each tensile test, since the initial condition
is based on the muscle-tendon complex length. As a consequence, the results regarding the velocity effect may be affected by the initial loadings.

$F_{\text{max}}$ is sensitive to changes in experimental conditions. $F_{\text{max}}$ decreases by 15% on average for a change from ambient conditions to immersion in cold solution, 8% when the cold saline solution is then heated and finally by 27% when the muscle passes from ambient conditions to immersion in heated saline solution. To our knowledge, no studies have evaluated the influence of experimental conditions (air and immersion) on the mechanical properties of muscles. However, the effect of temperature was studied. In [21], muscles of rabbits were tested in tension at 10 mm.s$^{-1}$ and 100 mm.s$^{-1}$. The temperature of the muscle of the right leg was set to 40°C before testing, whereas the muscle of the left leg had a temperature of 25°C. Load at failure and stiffness calculated on the linear part of the load-displacement curve were studied. The load at failure for muscles at 25°C was higher than that measured for muscles at 40°C regardless of the velocity applied (decrease of 8% to 15% for the load at failure between 25°C and 40°C). This result is also found in our study for $F_{\text{max}}$ between immersion in 22°C saline solution and in 35°C saline solution (decrease of 8%).

The stiffness measured between 7 and 8 mm displacement is not sensitive to velocity changes. However, it is sensitive to changes in experimental conditions. $K$ decreases by 15% between ambient conditions and cold solution, by 6% between cold solution and heated solution and by 27% between ambient conditions and heated solution. The percentages are similar to those calculated for $F_{\text{max}}$, and the results of the effect of temperature are consistent with the results provided in [21] in which they found that muscle stiffness at 25°C was decreased by 11% to 15% when muscle temperature was 40°C.

Even though only twelve muscles were tested, a high variability is present in the results (Fig. 4, Fig. 5). This variability is due to the parameters studied that are geometry-dependent and to inter-individual variability. However, the trend regarding the experimental conditions effect was shown for the two parameters and observed for each muscle studied independently. Nevertheless, this experimental conditions effect should be balanced. As for the velocity effect, a lot of tests were performed on the same muscle and thus, plastic strain may have appeared in the tendinous part leading to a decreased maximum load and stiffness.

V. CONCLUSIONS

In vitro tensile tests were performed on extensor carpi ulnaris muscles of dogs. The objective of these experiments was to study the influence of velocity and the influence of experimental conditions on muscle passive response. Velocities imposed were 1, 10 and 100 mm.s$^{-1}$, and muscles were tested in ambient conditions, in a saline solution at room temperature (22°C) and in a heated saline solution (35°C).

The velocity had no significant effect on the mechanical parameters in this study due particularly to the small range of velocities studied. Environmental conditions had a significant effect on muscle passive response (decrease of 15% to 27% of the maximum load and by 15% to 45% of the stiffness for a change from ambient conditions to immersion) and they must be taken into account in the definition of the mechanical properties used in modeling to get closer to reality.

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VII. REFERENCES


