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Research paper

Measurement of changes in muscle viscoelasticity during static stretching using stress-relaxation data

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ABSTRACT

This study investigates how muscle viscoelasticity changes during static stretching by measuring the state of the muscle during stretching using continuous time-series data. We used a device that applied a force to the muscle during stretching and measured the reaction force. The device was attached to each participant, and time-series data of the reaction force (stress-relaxation data) during stretching were obtained. The spring-pot model, which uses fractional calculus, was selected as the viscoelastic model for the muscle, to which the stress relaxation data were fitted on a straight line on a double-logarithmic plot. The stress-relaxation data formed a broken line comprising two segments on the double-logarithmic plot, showing that viscoelasticity changed abruptly at a particular time during static stretching. Considering two viscoelastic states, before and after the change, the stress-relaxation curve was fitted through segmented regression to the double-logarithmic data with high accuracy ($R^2 = 0.99$ and $\text{NRSME} = 0.0018$). We compared the parameters of the spring-pot model before and after the change in muscle viscoelasticity. By examining these continuous time-series data, we also investigated the time taken for the effects of stretching to become apparent. Furthermore, we measured the changes in muscle viscoelasticity during static stretching before and after a short-term exercise load of running on a treadmill.

1. Introduction

With the recent increase in health and fitness awareness, stretching is considered an important factor for improving health and athletic performance. For athletes and fitness-conscious individuals, stretching muscles after exercise is necessary to relieve fatigue and prepare them for the next workout. In addition, stretching is important for preventing muscle soreness and injuries that can occur after exercise. Stretching after exercise promotes blood flow and the flexibility of stiff muscles. Maintaining flexible muscles reduces strain on muscles and joints and prevents muscle and joint injuries. In modern society, the working population is aging and the physical burden on workers is increasing. Stretching is also important for reducing the physical burden on workers and in preventative medicine. Thus, the importance of stretching is multifaceted in today's increasingly health-conscious society. Therefore, it is important to investigate in detail how stretching changes muscles that have been stiffened by exercise.

Related research describes the changes in muscle properties due to stretching and exercise and changes in muscle condition during stretching. First, studies on the changes in muscle properties induced by stretching will be explained. Magnusson et al. investigated passive torque and electromyographic activity with static stretching and

reported that 90 s of stretching resulted in a decrease in passive torque (Magnusson et al., 1996). Mizuno et al. measured significant decreases in the stiffness of muscle-tendon units following rest periods of 0, 15, 30, 60, and 90 min after they were stretched for 60 s (Mizuno et al., 2013). Reisman et al. found that the passive torque on plantar flexor muscles increased and passive tension decreased with stretching (Reisman et al., 2009).

Next, we present studies that investigated changes in muscle properties owing to exercise. Murayama et al. reported that elbow flexor stiffness increased significantly after exercise (Murayama et al., 2000). Yanagisawa et al. showed that muscle hardness increased immediately after exercise and decreased 30 min later (Yanagisawa et al., 2011). By contrast, Niitsu et al. showed that muscle hardness increased after exercise, peaked on the second day after exercise, continued to increase, and then decreased (Niitsu et al., 2011). Hirono et al. reported that medial gastrocnemius muscle hardness increased significantly on days 3, 4, and 5 of an athletic-team training camp, and the joint range of motion decreased significantly on days 4 and 5 (Hirono et al., 2016).

Finally, we present studies that investigated the changes in muscle status during stretching. Ryan et al. measured joint angles during repeated stretching of the right plantar flexor muscles under constant

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torque. During 30 s of low-torque stretching, the right-ankle angle increased, which was considered to be viscoelastic creep (Ryan et al., 2010). Gajdosik et al. measured the torque generated on an ankle held at three dorsiflexion angles corresponding to 100%, 90%, and 80% of the maximum dorsiflexion force for 60 s (Gajdosik et al., 2006). The results showed that the ankle torque decreased, which was attributed to stress relaxation due to viscoelasticity.

Thus, previous studies have reported that stretching changes muscle properties and decreases hardness (Magnusson et al., 1996; Mizuno et al., 2013; Reisman et al., 2009). Exercise has also been reported to change muscle properties and stiffness (Murayama et al., 2000; Yanagisawa et al., 2011; Niitsu et al., 2011; Hirono et al., 2016). Furthermore, studies on muscle changes during stretching have reported phenomena related to muscle viscoelasticity (Ryan et al., 2010; Gajdosik et al., 2006). Most studies have measured muscle properties before and after stretching. Many of these studies did not measure muscle properties directly, but examined changes in joint range of motion or passive torque as indirect changes in muscle properties. Few of these studies measured muscles continuously and examined how muscle properties change during stretching. The stretching duration varied among studies, and no consistent view of the minimum stretching time required to achieve an effect was obtained.

Therefore, the objective of this study is to investigate how muscle viscoelasticity changes during static stretching by measuring the state of the muscle during stretching using continuous time-series data. By examining these continuous time-series data, we also investigate the time required for the effects of stretching to become apparent. Furthermore, we measure the changes in muscle viscoelasticity during static stretching before and after a short-term exercise load of running on a treadmill.

2. Methods

This study was approved by the Ethical Committee for Human Studies of the Graduate School of Engineering Science, The University of Osaka, Japan. All experiments were performed in accordance with the approved design. Before the experiment, all the participants signed an informed-consent form. The participants included in the figures gave informed consent for the publication of their images online.

In this study, the changes in muscle viscoelasticity were continuously captured by measuring the muscle reaction force during static stretching. We used a device that applied a force from the muscle during stretching and measured the reaction force. The device was attached to each participant, and time-series data of the reaction force during stretching were obtained. Time-series data of the measured reaction force were applied to a spring-pot model to identify the model parameters before and after the change in viscoelasticity. The changes in identified parameters of muscle viscoelasticity were measured during static stretching. To investigate the influence of short-term exercise on the results, measurements were obtained twice, before and after exercise. The participants were subjected to a 10-min treadmill run between the two measurements to provide an exercise load.

2.1. Experimental setup

The following describes the apparatus used to measure the muscle reaction force. Fig. 1 shows the device used to measure the reaction force of the muscle (rectus femoris). The reaction force from the muscle was obtained by pushing the central projection (indenter) of the device into the muscle. The indenter was a cylinder with a diameter of 9 mm that was in contact with the muscle and had a chamfer with radius $R = 4.5$ mm. A small compression-load cell (UNSCR-10N, Unipulse Corporation, Japan) was attached to the base of the indenter to measure the force applied to the indenter. The base of the load cell was attached to the base of the device, which was curved to prevent application of force to the muscle by the base. A non-stretchable band was attached to

the left and right sides of the base of the device and wrapped around the middle of the participant's thigh. As the muscle was enlarged by static stretching, the indenter of the device was pressed against the thigh muscle, and the reaction force was measured. The amount of muscle enlargement under static stretching (equal to the pushing displacement of the indenter) is expressed as ϵ_0 .

2.2. Protocol

Six healthy males with an average age of 24.1 years, daily exercise habits, and no history of injury or pain in the thigh muscles, including the rectus femoris, were tested. Each participant was tested an average of five times (with a standard deviation of 3.4) for a total of 30 times for the entire group. The experimental protocol is shown in Fig. 1(d) and was as follows.

Prior to the start of the experiments, the participants were instructed to perform their normal daily routines and were not given any special exercise load. In the first experiment, changes in muscle viscoelasticity were measured during static stretching [measurement (a)]. First, a non-contractile band was used to attach the device such that the indenter was placed in the middle of the thigh. Static stretching was then performed, during which the reaction force was measured at a sampling frequency of 1000 Hz for 90 s. The stretching period in this study was 90 s as in previous studies (Magnusson et al., 1996, 1995) on the effects of static stretching. Measurements were performed for both the left and right legs.

A treadmill (MARCHERFT-011, Fujimori, Japan) was used to provide the participants with an exercise load via running. The treadmill speed was manually adjusted such that the participant's heart rate was maintained at the target heart rate. The heart rate during running was measured using a fitness tracker (Fitbit Inspire HR, Fitbit, USA) attached to the participant's arm. The target heart rate was calculated with the Karvonen method (Robergs and Landwehr, 2002) using the following formula: target heart rate = $0.7 \times (\text{maximum heart rate} - \text{resting heart rate}) + \text{resting heart rate}$. The target heart rate was defined as the value at 70% exercise intensity. Various methods for calculating the maximum heart rate exist (She et al., 2015); however, we used the simplest method: maximum heart rate = $220 - \text{age}$. The resting heart rate of each participant was measured using the fitness tracker. The participants first warmed up by running at 6.2 km/h for 5 min and then ran on the treadmill for 10 min to maintain their target heart rate, which was followed by 5 min of cool-down at 5 km/h. The exercise load and speed of the treadmill during warm-up and cool-down were based on the treadmill speeds used in previous studies (De Weijer et al., 2003; Wiles et al., 1992; Morin and Sève, 2011).

At the end of the treadmill run, when the participant's breathing had calmed for approximately 5 minutes, an experiment was conducted to measure the change in muscle viscoelasticity during the static stretch [measurement (b)]. The method used in this experiment was the same as that used in the previous experiment before the treadmill run. Measurements were conducted for each leg, and data were obtained for both the left and right feet. Thus, the experiment to measure the change in muscle viscoelasticity under static stretching was performed twice, before and after running; the first and second measurements are referred to as "measurement (a)" and "measurement (b)", respectively. Measurement (a) was on the change in muscle viscoelasticity during static stretching when each participant was not intentionally exercising. Measurement (b) shows the short-term post-exercise (treadmill running) change in muscle viscoelasticity during static stretching.

2.3. Viscoelastic model

This section presents the viscoelastic model for muscle for analyzing stress-relaxation data. Muscle is a viscoelastic material (Fung, 2013) that exhibits a mixture of elasticity, in which the strain is proportional to stress, and viscosity, in which the strain rate is proportional to

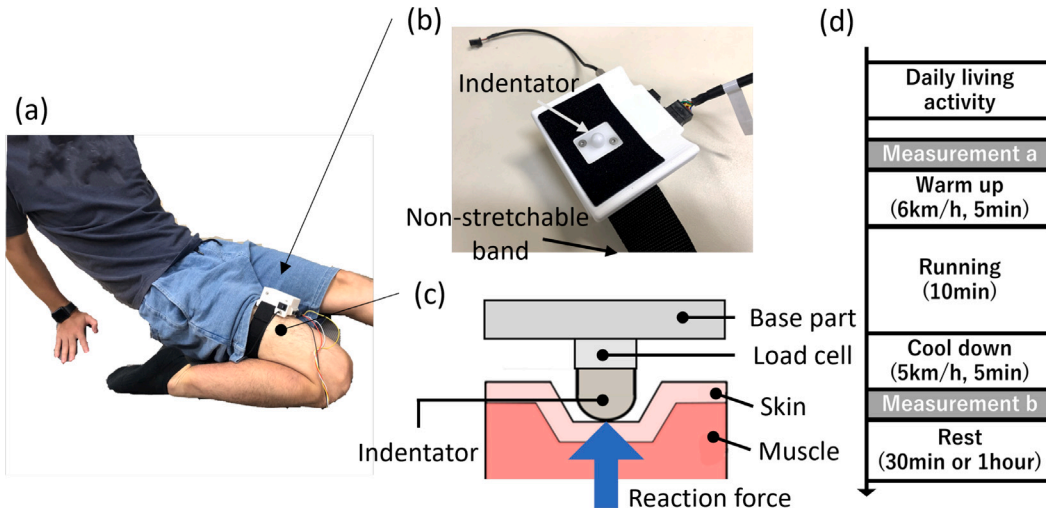


Fig. 1. Experimental setup: (a) Picture during static stretching. A non-contractile band was used to attach the device so that the indenter was placed in the middle of the thigh. (b) The device obtained the reaction force from the muscle by pushing the central projection (indenter) of the device into the muscle. (c) A small compression-load cell was attached to the base of the indenter to measure the force applied to the indenter. The base of this load cell was attached to the base of the device. (d) Experimental protocol.

stress. When a certain amount of strain is applied to a material and the material is held in place, stress is generated owing to the displacement of the molecules inside the material from their equilibrium positions if the material is elastic, whereas no stress is generated because of permanent deformation if the material is viscous. However, in a viscoelastic body, stress gradually decreases as the molecules flow internally. This is called stress relaxation and is a characteristic of viscoelastic materials (Fung, 2013). In general, a material is more viscous when the relaxation time is longer and more elastic when the relaxation time is shorter. In this study, we measured the stress relaxation that occurs when a certain amount of strain is applied to the muscle and considered the change in the stress trend to be a change in muscle viscoelasticity.

Biological tissues are complex viscoelastic bodies (Bilston, 2018). Classical viscoelastic models such as the Kelvin-Voigt model, standard linear solid model, and generalized Voigt model (Flügge, 1975), which consist of springs and dashpots connected in series or parallel, require many elements and parameters; thus, they are difficult to interpret accurately (Freed and Diethelm, 2006; Bonfanti et al., 2020). Therefore, models using fractional calculus called “springpot models” are often used to represent the viscoelasticity of biological tissues with fewer parameters (Zhang et al., 2018; Meral et al., 2010; Zhang et al., 2007; Kiss et al., 2004; Kobayashi et al., 2012; Tsukune et al., 2015; Kobayashi et al., 2017, 2020). An infinite number of springs and dashpots converge to a single element called a springpot, for which fractional calculus is used (Kobayashi et al., 2017). The springpot model can accurately represent viscoelasticity, which is characterized by the power law, with a small number of parameters (Freed and Diethelm, 2006; Bonfanti et al., 2020). In this study, a springpot model was selected as the viscoelastic model for the muscle because it can capture characteristics with a small number of parameters to easily capture changes during static stretching.

In the spring-pot model, the relationship between stress $F(t)$ (reaction force from the muscle) and strain $\epsilon(t)$ (indentation displacement), where t is time, can be written using fractional calculus as

$$F(t) = G\tau_0^r \frac{d^r \epsilon(t)}{dt^r}, \quad (1)$$

where G and r are parameters that characterize viscoelasticity. r determines the rate of stress relaxation, where a viscoelastic body is more elastic as r approaches 0 and more viscous as r approaches 1. G determines the magnitude of the force scale at a certain time τ_0 during the stress relaxation. τ_0 denotes the reference time scale.

Given a constant strain $\epsilon(t) = \epsilon_0$ at time $t > 0$, force $F(t)$ is given as (Bonfanti et al., 2020)

$$F(t) = \frac{G\epsilon_0}{\Gamma(1-r)} \left(\frac{t}{\tau_0} \right)^{-r}, \quad (2)$$

where $\Gamma()$ is the gamma function. The log transformation of both sides of Eq. (2) yields the following equation, where $\log F(t)$ is a linear function of $\log(t)$:

$$\log F(t) = -r \log \left(\frac{t}{\tau_0} \right) + \log \left(\frac{G\epsilon_0}{\Gamma(1-r)} \right). \quad (3)$$

Thus, in the stress-relaxation data, if the viscoelastic properties (parameters G and r in this model) do not change during static stretching, then plotting the relationship between time t and reaction force $F(t)$ on a double-logarithmic graph yields a straight line. A change in the slope or intercept of the line indicates a change in the parameters r and G that characterize viscoelasticity, thereby indicating a change in muscle viscoelasticity.

This study assumed two types of muscle states, one before and one after the change due to static stretching. In this case, the regression line of the stress relaxation data is divided into two segments on a double-logarithmic graph according to the equation

$$\begin{aligned} \log F(t) &= -r_1 \log \left(\frac{t}{\tau_0} \right) + \log \left(\frac{G_1 \epsilon_0}{\Gamma(1-r_1)} \right) & \{t < t_c\} \\ \log F(t) &= -r_2 \log \left(\frac{t}{\tau_0} \right) + \log \left(\frac{G_2 \epsilon_0}{\Gamma(1-r_2)} \right) & \{t > t_c\}, \end{aligned} \quad (4)$$

where r_1 and G_1 are the viscoelastic parameters before stretching, r_2 and G_2 are the viscoelastic parameters after stretching, and t_c is the break point of time before and after stretching.

2.4. Data processing

This section describes the data-processing methods used to calculate the model parameters (G_1 , G_2 , r_1 , and r_2) in Eq. (4) from the time-series data of the muscle reaction forces.

The time at which the force on the indenter is at its maximum was set to $t = 0$ [s]. The reaction forces were expressed as percentages and set to 1 at $t = 1$ [s] to focus on the relative changes.

Then, to smooth the acquired data, a moving average was taken from the obtained reaction-force time-series data with an interval of 500 [ms]. The data were then resampled so that they were equally spaced on double-logarithmic plots.

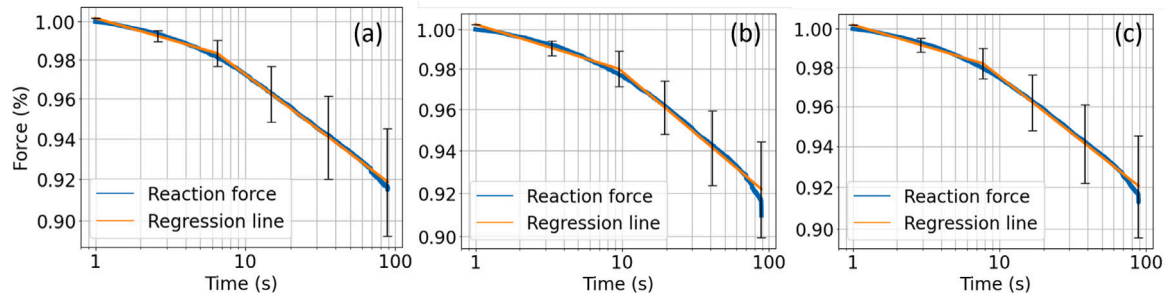


Fig. 2. Double-logarithmic graph of the time series of the averaged reaction force (stress-relaxation data) during static stretching and segmented regression line of each experimental dataset. In each figure, the blue line shows the experimental result, and the orange line shows the segmented regression line. The error bars indicate one standard deviation. (a) Data obtained in measurement (a), (b) data obtained in measurement (b), and (c) data of both measurements (a) and (b) combined. In (a), (b), and (c), the reaction force is expressed as a percentage and is set to 1 at $t = 1$ [s] to focus on relative changes. The mean forces at $t = 1$ [s] for (a), (b), and (c) were 10.4, 11.5 and 10.9 [N], respectively. Considering the two viscoelastic states, before and after the change, each double-logarithmic stress-relaxation curve consists of two segments fitted with high accuracy. The coefficient of determination (R^2) is = 0.99, and the normalized root mean square error (NRMSE) is 0.0018.

This study assumed two types of muscle states, one before and one after the change due to static stretching. Thus, we divided the data into two segments and calculated the parameters from the slopes and intercepts of the two regression segments. Segmented regression (Muggeo, 2003) was performed on the resampled data on double-logarithmic plots to obtain the slopes, intercepts, and break point t_c of the two-segment line.

From the slope and intercept of each straight regression segment, we calculated the parameters r_1 , r_2 , G_1 , and G_2 using Eq. (4). From Eq. (4), the parameter r_1 is the slope of the line before the stretching and r_2 is the slope of the line after the stretching. Parameters G_1 and G_2 can be calculated using $G = I_o F(1 - r)/\epsilon_0$, where I_o is the regression-line value at $t = \tau_0$. Therefore, τ_0 is related to G_1 and G_2 . In this study, we set $\tau_0 = 1$ for simplicity. This implies that on a double-logarithmic plot, the point at which the stress-relaxation line intersects the vertical axis of $t = 1$ [s] is the standard for the magnitude of G . The value of ϵ_0 in Eq. (4) corresponds to the amount of muscle enlargement (equal to the displacement of the indenter when pushed into the muscle). Because this value cannot be measured owing to the experimental method used in this study, we set $\epsilon_0 = 1$. Note that in the results and discussion that follow, we focus only on the relative changes between the parameters G_1 and G_2 ; thus, setting $\epsilon_0 = 1$ has no effect on the results or discussion of this study.

The reaction force of the muscle increased instantaneously during a voluntary contraction of the participant's muscle during measurement. To exclude voluntary contractions from the experimental data, the data were discarded on the basis of a coefficient of determination of $R^2 > 0.9$ for the results obtained by segmented regression. An example of discarded data is shown in Appendix B.

The Mann-Whitney U test was also performed on the parameters obtained from the data of the left and right feet, each at a significance level of $p < 0.05$. No significant difference was observed between the results for the left and right feet in measurements (a) and (b). Therefore, the left-right foot differences were not considered for the given measurement data.

Hereafter, the parameters from the first regression segment at measurement (a) are defined as r_1^a and G_1^a , and the parameters for the second regression segment as r_2^a and G_2^a . The boundary point between the two segments and time at which the stress-relaxation trend changed was defined as t_c^a . Similarly, in measurement (b), we defined the parameters of the first regression segment as r_1^b and G_1^b , those of the second regression segment as r_2^b and G_2^b , and the time of the segment boundary point as t_c^b . In addition, in the mixed data of measurements (a) and (b), we defined the parameters of the first regression segment as r_1^{ab} and G_1^{ab} , those of the second regression segment as r_2^{ab} and G_2^{ab} , and the time of the segment boundary as t_c^{ab} .

2.5. Statistical analysis

The Wilcoxon signed-rank test (Woolson, 2007) was performed on the viscoelastic-model parameters r_1^a and r_2^a obtained from measurement (a) and on r_1^b and r_2^b obtained from measurement (b), both at a significance level of $p < 0.05$. A Wilcoxon signed-rank test was also performed for r_1^{ab} and r_2^{ab} by combining the results of measurements (a) and (b) at a significance level of $p < 0.05$. Wilcoxon signed-rank tests were also conducted for the parameter-change points t_c^a and t_c^b at a significance level of $p < 0.05$.

The correlation coefficients for each parameter were calculated from measurement results of (a) and (b). The parameters considered were r_1 , r_2 , G_2/G_1 , $r_2 - r_1$, and r_2/r_1 . Each parameter and its change $r_2 - r_1$, change rate G_2/G_1 , and r_2/r_1 were considered as variables. In this correlation analysis, the data for measurements (a) and (b) were not separated, but all the data (r_1^{ab} , G_1^{ab} , r_2^{ab} , and G_2^{ab}) were used.

3. Results

3.1. Stress-relaxation test

Fig. 2(a) shows a double-logarithmic graph of the time series of the averaged reaction force during a stress-relaxation test ($N=30$) obtained in measurement (a). The data obtained from measurement (b) are shown in Fig. 2(b), and the data of both measurements (a) and (b) combined ($N=60$) are shown in Fig. 2(c). Fig. 2 shows each set of experimental data and the segmented regression line fitted to it. In this experiment, ϵ_0 was the amount by which the muscle was enlarged (equal to the displacement of the indenter pushed into the muscle). Because the exact value of ϵ_0 could not be determined in each experiment, the absolute values of G could not be compared between each measurement. However, if the relative value G_2/G_1 is considered, the term ϵ_0 disappears, and comparisons can be made between the measurements. Note that in Fig. 2(a), (b), and (c), the reaction forces are expressed as percentages and set to 1 at $t = 1$ [s] to focus on the relative changes. The experimental results in Fig. 2 show that the stress-relaxation curves (double-logarithmic plots) each comprise two segments. Each stress-relaxation curve was fitted to the experimental results with high accuracy using segmented regression. Moreover, the coefficient of determination (R^2) for the fitted regression line is high (0.99), and the normalized root mean square error (NRMSE) is low (0.0018).

3.2. Parameter changes

For parameters r_1 and r_2 , the box-and-whisker diagrams for measurement (a) are shown in Fig. 3(a), those for measurement (b) in Fig. 3(b), and those for the combined data of measurements (a) and

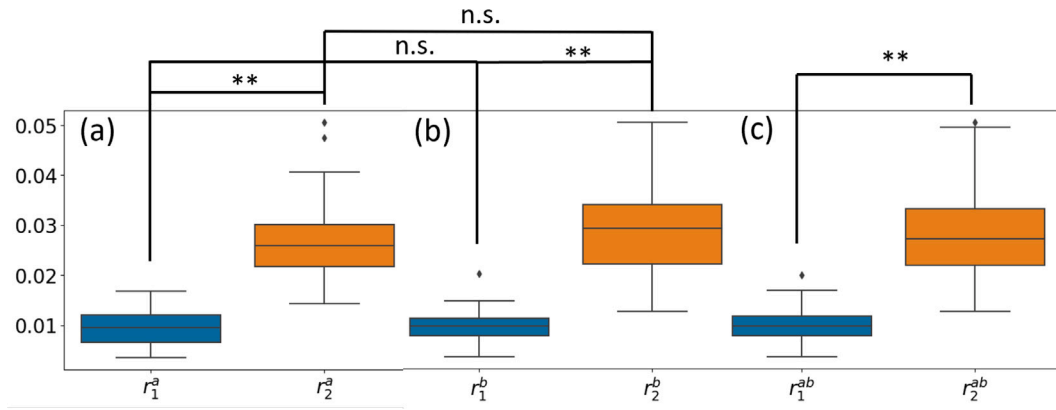


Fig. 3. Box-and-whisker diagrams of parameter r obtained for (a) measurement (a), (b) measurement (b), and (c) the combined data of measurements (a) and (b). The statistical analysis showed that r_2 increased significantly with respect to r_1 in the data for measurement (a), measurement (b), and measurements (a) and (b) combined (**: $p < 0.01$).

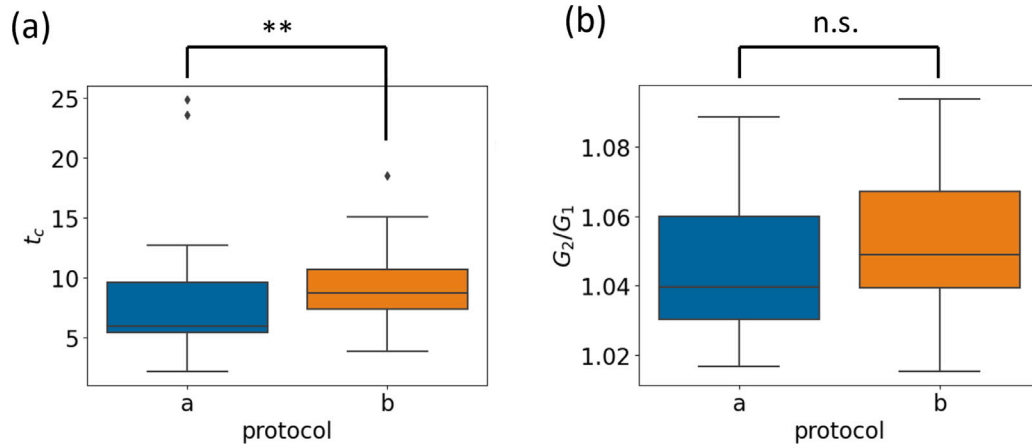


Fig. 4. Box-and-whisker diagrams of parameters t_c and G_2/G_1 obtained for measurements (a) and (b). (a) The time required for t_c to change increased significantly (**: $p < 0.01$) in measurement (b) compared to measurement (a). (b) No significant difference was observed for the relative value G_2/G_1 between the data in measurements (a) and (b).

(b) in Fig. 3(c). The Wilcoxon signed-rank test, used for the statistical analysis, showed that r_2 increased significantly with respect to r_1 for measurement (a), measurement (b), and both measurements (a) and (b) combined (**: $p < 0.01$).

Fig. 4(a) shows a box-and-whisker plot comparing the time required for the parameter changes (t_c) in measurements (a) and (b). The Wilcoxon signed-rank test for measurements (a) and (b) shows that t_c increased significantly (**: $p < 0.01$) in measurement (b) compared to measurement (a).

Fig. 4(b) shows a box-and-whisker plot comparing the relative value G_2/G_1 of the G parameters for measurements (a) and (b). Parameter G cannot be compared between different measurements because ϵ_0 , the amount of indentation into the muscle, cannot be measured. Therefore, the relative value G_2/G_1 was examined. A Wilcoxon signed-rank test was performed on the G_2/G_1 results for measurements (a) and (b), and no significant differences were observed between them ($p = 0.12$). Similarly, there were no significant differences between measurements (a) and (b) for r_1 and r_2 (r_1 : $p = 0.84$; r_2 : $p = 0.40$), as shown in Fig. 3. Therefore, in the results that follow, the results of measurement (a) are not separated from those of measurement (b). Instead, the results are presented for the parameters G_2^ab/G_1^ab , r_1^ab , and r_2^ab , which combine both datasets.

3.3. Correlations among parameters

Table 1 lists the correlation coefficients of each parameter. The following figures show strong correlations among the parameters. Fig. 5(a) shows a scatter plot and histogram of the parameters r_2^ab and

Table 1

Correlation coefficients between the parameters.

	r_1^ab	r_2^ab	$r_2^ab - r_1^ab$	r_2^ab / r_1^ab	G_2^ab / G_1^ab
r_1^ab	–	0.756	0.472	–0.534	0.426
r_2^ab	–	–	0.934	0.090	0.830
$r_2^ab - r_1^ab$	–	–	–	0.413	0.885
r_2^ab / r_1^ab	–	–	–	–	0.324
G_2^ab / G_1^ab	–	–	–	–	–

$r_2^ab - r_1^ab$ with a correlation coefficient of 0.934. Fig. 5(b) shows a scatter plot and histogram of the parameters G_2^ab/G_1^ab and $r_2^ab - r_1^ab$ with a correlation coefficient of 0.885. Correlation coefficients between r_2^ab and G_2^ab/G_1^ab and between r_2^ab and r_1^ab were also high (0.83 and 0.756, respectively).

4. Discussion

To date, only a few studies have measured viscoelasticity changes during static stretching, and these changes remain largely unknown. The main contribution of this study is to show that viscoelasticity changes abruptly at a certain time during static stretching because the stress-relaxation data can be accurately fitted by segmented regression on double-logarithmic plots. The change in muscle viscoelasticity during static stretching was captured by fitting a stress-relaxation curve consisting of two line segments to double-logarithmic data via the spring-pot model. Considering the two viscoelastic states, before and after the change, the stress-relaxation curve was fitted via segmented

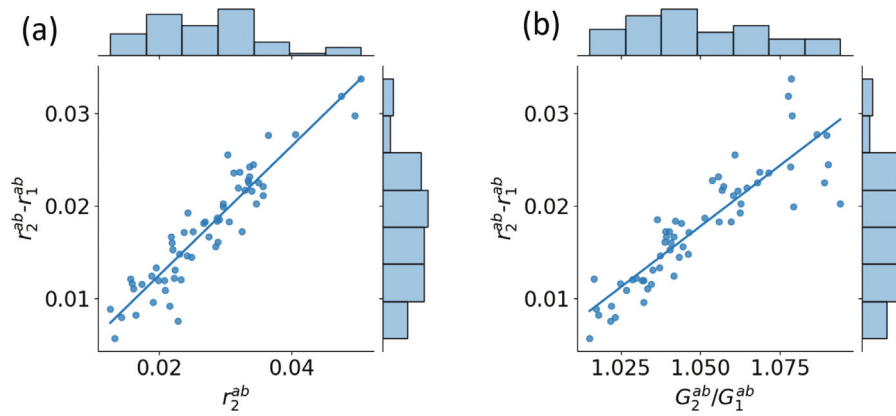


Fig. 5. Correlation coefficients for each parameter. (a) Scatter plot and histogram of parameters r_2^{ab} and $r_2^{ab} - r_1^{ab}$ with a correlation coefficient of 0.934. (b) Scatter plot and histogram of parameters G_2^{ab}/G_1^{ab} and $r_2^{ab} - r_1^{ab}$ with a correlation coefficient of 0.885.

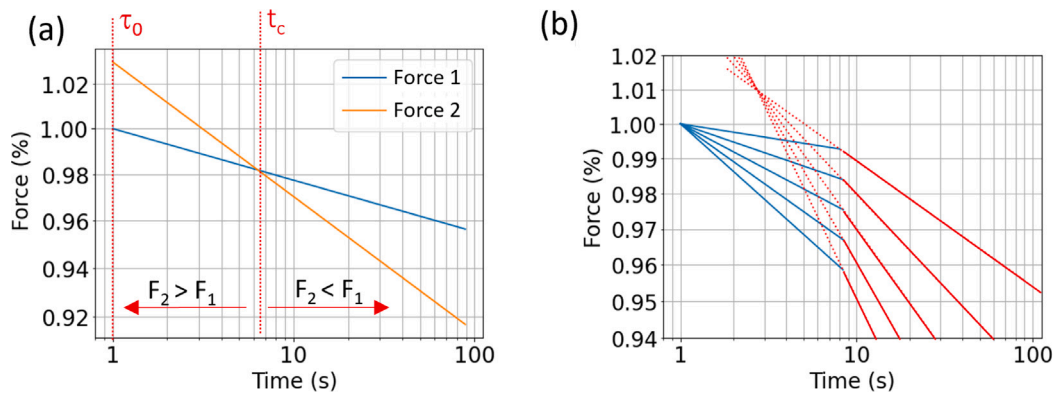


Fig. 6. (a) Comparison of muscle reaction forces before and after the viscoelasticity change. (b) Example of a stress-relaxation test from constraints on parameters: If the muscle state at the start of static stretching (blue line) is known, the state at the end (red line) can be predicted. Each line after the change in viscoelasticity due to static stretching (red line) is extrapolated back with a dotted line. The lines showing the stress relaxation after the change intersect at approximately one point. A similar tendency was observed in research on various cells (Kollmannsberger and Fabry, 2011).

regression with high accuracy. The coefficient of determination of the fitted regression line is high ($R^2 = 0.99$ and $\text{NRMSE} = 0.0018$).

An increase in the parameter r in the spring-pot model induced a faster stress relaxation. Conversely, a decrease in r resulted in a slower stress relaxation. Parameter r increased during static stretching in both measurements (a) and (b). Therefore, static stretching is expected to induce faster stress relaxation.

Measurements (a) and (b) show that $G_2/G_1 > 1$ and G increased during static stretching. However, this does not mean that static stretching increased “muscle hardness” when simply defined as “muscle hardness = reaction force/indentation displacement”. Parameter G varied with τ_0 in the spring-pot model. The value of G_2/G_1 also changed with τ_0 ; $G_2/G_1 > 1$ for $\tau_0 < t_c$ and $G_2/G_1 < 1$ for $\tau_0 > t_c$. Most studies have reported a decrease in muscle hardness after static stretching (Mizuno et al., 2013; Reisman et al., 2009; Magnusson et al., 1996). However, to avoid the effects of stress relaxation, those studies measured muscle hardness via the reaction force after sufficient time was taken to measure the muscle hardness (Mizuno et al., 2013; Reisman et al., 2009; Magnusson et al., 1996). This is equivalent to measuring when $\tau_0 > t_c$, i.e., $G_2/G_1 < 1$ in the results of this study. In fact, comparing the regression lines before and after the change in this study shows that the simple reaction force was strong after the change before t_c , the time before the viscoelasticity changed, as shown in Fig. 6(a). However, after t_c , the value after the change decreased. Therefore, after a time lapse of t_c , the simple muscle hardness decreased. Thus, the results of this study do not conflict with those of previous studies that reported a decrease in muscle hardness after static stretching.

The clinical significance of this study is finding that viscoelasticity does not gradually change, but changes abruptly at a certain time

during static stretching. Another clinically significant achievement of this study is defining the specific number of seconds required to change muscle characteristics during static stretching. The experimental results also showed that the time required for the muscle viscoelasticity to change during static stretching significantly increased after treadmill running. In measurement (a), i.e., when the participants performed only daily movements before the measurement, the change in the muscle viscoelasticity owing to static stretching was observed after 6.5 s. Measurement (b), performed after short-term exercise, required approximately 9.5 s. This suggests that the time required to extend the muscle by static stretching is longer if the characteristics of the muscle have changed when it has been stiffened by exercise. Note that prior to the start of the experiments in this study [before measurement (a)], participants were instructed to go about their normal routine and were not given any specific exercise load. The results of measurements (a) and (b) suggest that static stretching for approximately 10 s was effective. The results of this study are consistent with those of previous studies. For example, according to Bandy et al. static stretching of the hamstrings for 30 s is necessary to increase the range of motion of the hip joint (Bandy et al., 1997). They also reported that increasing the time from 30 to 60 s did not improve flexibility. In addition, Borms et al. reported that 10 s of static stretching was sufficient to improve the flexibility of the femoral neck, and no significant differences were observed between groups that performed 10, 20, or 30 s of static stretching (Borms et al., 1987).

In Fig. 5, the correlation between r_1 and $r_2 - r_1$ is high, suggesting that if the muscle state r_1 at the start of static stretching is known, the state r_2 at the end can be predicted. This suggests that static

stretching does not bring the muscle state back to some baseline, but rather changes it relative to the initial state. In addition, the correlation between G_1/G_2 and $r_2 - r_1$ was high. The results suggest certain constraints on the parameters when viscoelasticity changes. If this constraint is used and considered with respect to the pattern of stress relaxation during stretching, the final state can be estimated using the correlations once the state at the beginning of the static stretch is known. The process of this estimation is shown in Appendix A. Fig. 6(b) shows the stress-relaxation curve obtained using this constraint relationship.

In Fig. 6(b), each line after the change in viscoelasticity owing to static stretching (red line) is extrapolated back with a dotted line. Fig. 6(b) shows that the lines representing stress relaxation after the change intersect at approximately one point. A study measuring the viscoelasticity of cells (Kollmannsberger and Fabry, 2011) reported a similar tendency for lines to intersect at a point. Kollmannsberger and Fabry (2011) compared the creep properties of various cells and reported that, when their creep test results were extrapolated, straight lines intersected at a single point (Kollmannsberger and Fabry, 2011), which is similar to the results of this study. They reported that the cells may not be able to change their elastic and viscous properties independently. They further reported that the elastic-to-viscous ratio must change when the stiffness changes. Moreover, to increase in stiffness, a cell must become more solid (i.e., the parameter r must decrease), and to decrease in stiffness, it must become more fluid (i.e., r must increase). With respect to muscle properties after viscoelasticity changes, the results in Fig. 6(b) exhibit the same mechanism observed in cell viscoelasticity (Kollmannsberger and Fabry, 2011). In other words, some mechanisms change the ratio of elasticity to viscosity when the muscle stiffness changes, and the results of this study suggest that muscle viscoelasticity changes according to these mechanisms. In the study by Kollmannsberger and Fabry (2011), cell viscoelasticity was based on tests on cells with constant normal properties and not on changes in viscoelastic properties. Thus, this study suggests that the above mechanisms may also be present when muscle properties change.

The main limitation of this study is that there is no control. As stated above, the main contribution of this study is to find the abrupt change in stress relaxation during stretching, but no comparison of the stress relaxation of the muscles under the same conditions and without stretching has been carried out. This is because the muscle enlargement due to stretching is used as an input for stress relaxation, so it is not possible to obtain a control for the same condition. Therefore, strictly speaking, it is impossible to say whether the abrupt changes are the effect of stretching or the effect of the experimental setup applied to any muscle (or soft tissue). However, the stress relaxation curve of muscle tissue is a single-segment regression line (ref: Van Loocke et al., 2008) according to Eq. (3), if its properties are constant. Because the stress relaxation curve is not a one-segment regression line, this indicates that the muscle properties have been changed in some way.

The other main limitation of this study is that the muscle enlargement (indentation displacement) ϵ_0 cannot be measured and is assumed to be 1. This was unavoidable because of the nature of the experiment, in which the reaction force was measured using an indenter pushing against the thigh muscle as the muscle was enlarged by static stretching. Therefore, the overall force magnitude of the stress-relaxation data was not meaningful, and the force was expressed as a percentage, with the value at 1 s after the start of static stretching being 1. In addition, the absolute values of G_1 and G_2 are not meaningful, and only their relative value G_2/G_1 is considered. Moreover, the magnitude of G cannot be determined if it is correlated with other parameters. However, the inability to measure ϵ_0 does not affect the findings of this study.

CRediT authorship contribution statement

Yo Kobayashi: Writing – original draft, Methodology, Writing – review & editing, Funding acquisition, Supervision, Conceptualization. **Daiki Matsuyama:** Investigation, Methodology, Writing – review & editing, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Parameter constraints

The constraint relationships between r_1 and $r_2 - r_1$ and between G_1/G_2 and $r_2 - r_1$ are discussed below with respect to the stress-relaxation pattern during stretching. The relationship between G_1/G_2 and $r_2 - r_1$ is obtained using the least-squares method as

$$\frac{G_2}{G_1} = 2.98 \times (r_2 - r_1) + 0.997. \quad (5)$$

Using the least-squares method for r_1 and r_2 , we obtain the following equation:

$$r_2 = 1.86r_1 + 0.009 \quad (6)$$

In this study, the absolute value of G cannot be considered because ϵ_0 , the displacement through which the indenter is pushed into the muscle, is unknown. Therefore, $G_1 = 1$ is assumed. We assume that $G_1 = 1$ is equivalent to expressing the force as a percentage, with the value at 1 s after the start of the static stretch being 1. Assuming $G_1 = 1$, determining any r_1 value yields G_2 and r_2 from Eqs. (5) and (6), respectively. In other words, if the state at the beginning of the static stretch (r_1) is known, the final states (G_2 and r_2) can be estimated using the constraints from the correlations. We selected r_1 values and calculated G_2 and r_2 using Eqs. (5) and (6), respectively, obtaining the results in Fig. 6(b).

Appendix B. Example of discarded data

The reaction force of the muscle increased instantaneously during a voluntary contraction of the participant's muscle during measurement. To exclude voluntary contractions from the experimental data, the data were discarded on the basis of a coefficient of determination of $R^2 > 0.9$ for the results obtained by segmented regression. An example of discarded data is shown in Fig. 7.

Data availability

Data will be made available on request.

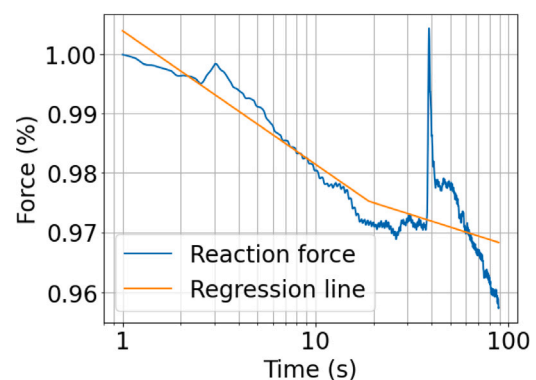


Fig. 7. Example of discarded data.

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