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ORIGINAL ARTICLE

Passive Mechanical Properties of Skeletal Muscle: Analyzing the Effects of Denervation with Mathematical Modelling in a Rabbit Quadriceps Model

Senol BEKMEZ[1*], [MD]
Akin UZUMCUGIL[2], [Associate Prof.]
Erkan KALAFAT[3], [MD]
Musa Ugur MERMERKAYA[4], [Assistant Prof.]
Nagehan DEMIRCI[5], [M.S.]
Ergin TONUK[5], [Assistant Prof.]
Gursel LEBLEBICIOGLU[2], [MD] [Prof.]

1 Dr. Sami Ulus Children's Hospital, Department of Orthopaedics and Traumatology
2 Hacettepe University Faculty of Medicine, Department of Orthopaedics and Traumatology
3 Ankara University Faculty of Medicine, Department of Obstetrics and Gynecology
4 Bozok University Faculty of Medicine, Department of Orthopaedics and Traumatology
5 Middle East Technical University, Department of Mechanical Engineering
* *Corresponding Author: Senol Bekmez, MD, Dr. Sami Ulus Children's Hospital, Department of Orthopaedics and Traumatology , Ankara, Turkey*
e-mail drsenolbekmez@gmail.com

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ABSTRACT

Background: The aim of the study was to analyze the effects of denervation on skeletal muscle in-vitro passive mechanical properties utilizing a fractional order viscoelastic material model.
Methods: 24 New Zealand rabbits were grouped into two; control group (n = 8) and denervation group (n = 16). In-vitro passive mechanical tests were performed on healthy and denervated quadriceps muscles. Relaxation and creep test curves were fitted with the best fitting curve of the 'three-element fractional viscoelastic material model'. Following this eight material parameters characterizing the passive mechanical material properties were extracted for each specimen (E_1, E_2, τ, β_r, $1/E_1$, $1/E_2$, η and β_c).
Results: The fractional order viscoelastic model demonstrated good curve-fitting to the experimental data and least square error values were found to be below 1%. There were statistically significant differences in two parameters for stress relaxation. Firstly, denervated skeletal muscles demonstrated a more solid material behavior in stress relaxation tests (β_r) ($p = 0.042$). Secondly, healthy muscles were relaxed significantly faster than denervated ones (τ) ($p = 0.017$). There was no significant difference between groups in creep tests.
Conclusions: Denervation altered some in-vitro stress-relaxation properties of the skeletal muscles but did not affect creep performance. The effects of pathological conditions in the passive mechanical properties of skeletal muscle can be analyzed with fractional order viscoelastic material models.

Key words: Passive Mechanical Properties, Skeletal Muscle, Denervation, Modelling.

Introduction

Skeletal muscle is composed of interconnected contractile and structural proteins, membranes and extracellular matrix elements. These elements constitute both load bearing and force production properties of the muscle. Load–deformation characteristics of the skeletal muscle are as important as contractile and motion generating properties, because passive mechanical behavior determines the load-bearing features [1,2]. Like most biological tissues, passive skeletal muscle has viscoelastic material behavior that exhibits time- and strain-dependent responses to tensile loads [3]. Thus, components of muscle viscoelasticity, such as muscle length, length extensibility, passive elastic stiffness, muscle mass and strength can be considered as potential therapeutic targets to achieve optimal muscle functions. Understanding the material properties of skeletal muscle is essential to develop management strategies for pathologic conditions such as injury, denervation or immobility.

Chronic denervation of skeletal muscle causes well-described histologic and morphologic alterations such as cellular atrophy, apoptosis, changes in microvascular architecture and deposition of connective tissue elements in intra- and extracellular spaces [4,5]. Eventually, these alterations in structural components induce some adaptations on physiologic length-extensibility and passive elastic stiffness of muscle such as decreased extensibility and increased stiffness [6,7]. Nevertheless, there is no quantitative analysis in the literature to describe the effects of denervation on the components of viscoelastic material behavior of skeletal muscle.

The aim of the study was to describe the effects of denervation on in-vitro, passive, time-dependent mechanical properties of the skeletal muscle in an experimental model based on force-elongation, using a non-integer order viscoelastic material model utilizing fractional calculus.

Materials and Methods

Approval was obtained from the local ethics committee for experiments on animals for this study. Quadriceps muscles of adult New Zealand rabbits were utilized to analyze the in-vitro passive load-deformation characteristics. Twenty-four male New Zealand rabbits were included into the study. Animals were anesthetized with a combination of ketamine and xylazine. All surgical procedures and the care of the animals were performed in the laboratory of experiments on animals in our institution. 24 adult rabbits were grouped into two;

Group 1 (control group) included 8 *six months-old male* rabbits. Following sacrification, bilateral hemipelvectomy was performed to isolate 16 quadriceps muscles with preservation of the pelvic and tibial bone insertions.

Group 2 (denervation group) included16 *two months-old* male rabbits. Femoral neurectomy was performed on the right side, under anesthesia. After four months, following sacrification, hemipelvectomy was performed on the right side to isolate the quadriceps muscle with preservation of the pelvic and tibial bone insertions. Contralateral limbs were not used as internal controls in the denervation group, because of the potential effects of denervation on the healthy weight-bearing extremity.

Patellar tendon reflex examination was used to confirm the permanence of total denervation on the quadriceps muscle at the follow-ups. Euthanasia was performed via peritoneal sodium pentobarbital injection.

Mechanical Analyzes

In-vitro passive mechanical analysis on skeletal muscles were performed at room temperature. Pilot studies have shown that rigor mortis occurs approximately one hour after isolation. Because of this, the mechanical analysis of the muscles were performed immediately after isolation to elude the corruptive effect of rigor mortis over the specimens.

Mechanical tests were performed with Zwick/Roell Z020 computer-controlled uni-axial mechanical testing system. The bony parts of the specimens were fixed to the specially designed and manufactured jigs of the testing system with K-wires. All non-destructive force relaxation and creep tests were performed for each specimen with a constant strain rate. Quadriceps muscles were adjusted to a quasi steady-state condition at the time before stretching in both force relaxation and creep tests. Pilot studies have demonstrated that a force around 65 Newtons causes permanent deformation on both healthy and denervated quadriceps muscles. Because of this, the maximum force was selected as 40 Newtons to simulate a load within the range of physiologic loads for the skeletal muscle.

Mathematical Modelling

In the viscoelastic approach, simpler linear models such as Kelvin, Voight, Maxwell and Zener models are assumed to behave linear under infinitesimal strains. However in reality, the majority of viscoelastic media, such as biological tissues, behave physically nonlinear. Therefore it becomes a necessity to analyze the viscoelastic behavior in the framework of nonlinear mechanics for both physical and geometrical nonlinearity.

Fractional calculus applied in the viscoelastic models comes from the idea that, for "intermediate" materials (in between fluid and solid) there should be a non-integer order time derivative relation between stress and strain. As *"spring"* element represents an ideal Hookean elastic solid and a *"dashpot"* (linear viscous damper) element represents a Newtonian viscous fluid, a new element called *"spring-pot"* represents an intermediate material between an elastic solid and a viscous fluid. The constitutive equation of the spring-pot as described by Bagley and Torvik is presented in Equation 1 [8].

$$\sigma\left(t\right)=E\eta^{\beta}\frac{d^{\beta}\grave{o}}{dt^{\beta}},\ 0\leq\beta\leq1 \quad (1)$$

As β assumes any real number in between 0 and 1, the spring-pot changes monotonically and continuously from an elastic solid ($\beta=0$) to a viscous fluid ($\beta=1$). In fractional order viscoelastic model representations, the dashpot in the linear integer order viscoelastic models is replaced with the *"spring-pot"* element.

For the *'three element fractional viscoelastic model'* (Figure 1) which is used to model the skeletal muscle viscoelastic behavior, Caputo and Mainardi generalized the integer order derivatives in the standard three element linear solid model to fractional order [9,10].

The constitutive equation of the model is presented in Equation 2.

$$\left(\frac{d^{\beta}}{dt^{\beta}}+\frac{\left(E_2+E_1\right)}{E_2\eta^{\beta}}\right)\sigma(t)=E_1\left(\frac{d^{\beta}}{dt^{\beta}}+\frac{1}{\eta^{\beta}}\right)\varepsilon(t) \quad (2)$$

Using this equation, the relaxation stiffness and creep compliance of the *'three-element fractional viscoelastic model'* can be obtained as following Equations 3 and 4, respectively;

$$G(t)=E_1\left\{1-\frac{E_1/E_2}{1+E_1/E_2}\left\{1-E_{\beta}\left[-\left(\frac{t}{\tau}\right)^{\beta}\right]\right\}\right\} \quad (3)$$

$$J(t)=\frac{1}{E_1}\left\{1+\frac{E_1}{E_2}\left\{1-E_{\beta}\left[-\left(\frac{t}{\eta}\right)^{\beta}\right]\right\}\right\} \quad (4)$$

$$\text{where } \tau=\frac{\eta}{\sqrt[\beta]{1+E_1/E_2}}.$$

The constitutive equation for the model involves four parameters to be extracted by experimental data and each parameter represents a specific viscoelastic property;

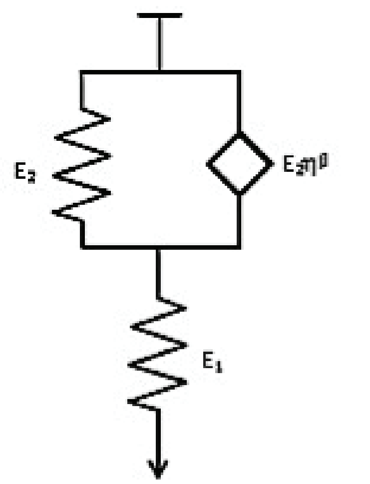

Figure 1. The schematical presentation of the 'three element fractional viscoelastic material model'. The diamond symbol represents the *'spring pot'*. (E_1, E_2 symbolize elastic modulus. η is time parameter. β is indicating where the spring-pot is in between an elastic solid or viscous fluid.)

$E_0=E_1+E_2$	N/mm, instantaneous elastic stiffness of the muscle;
E_1	N/mm, long-term elastic stiffness of the muscle;
τ, η	seconds, time constants representing the time at which the transition is centered for relaxation and creep, respectively.
b_r and b_c	non-dimensional parameters,indicating where the spring-pot is in between an elastic solid or viscous fluid ($\beta=0$ is full elastic solid, $\beta=1$ is full viscous fluid) for relaxation and creep, respectively. β determines the transition from glassy to rubbery behavior.

Using these mathematical equations, multiple force relaxation (Figure 2) and creep (Figure 3) curves can be obtained with a variable β parameter and constant E_1, E_2, η and τ parameters. The curve represents an elastic solid when $\beta=0$, and a viscous fluid when $\beta=1$ and an intermediate viscoelastic material when $0<\beta<1$.

Curve-Fitting

The experimental data obtained from the creep and relaxation tests were fitted with 'best-fitting' curves of the *'three element fractional viscoelastic model'* by using Matlab® optimization toolbox with Levenberg-Marquardt nonlinear optimization algorithm (Figures 4 and 5). For optimal curve fitting, nonlinear least squares algorithm was selected. By minimizing the LSE measure between the measured data and the model, the model parameters were identified optimally. Least squares error (LSE)

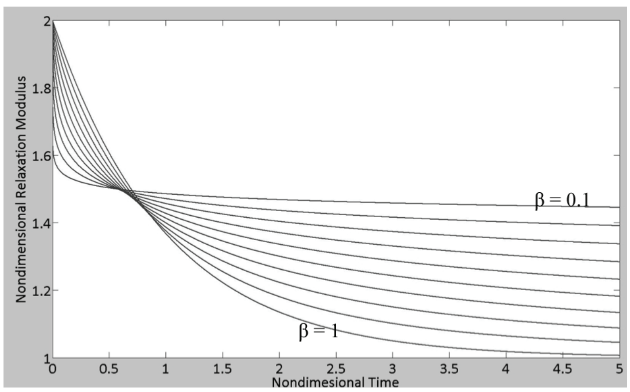

Figure 2. Multiple force relaxation curves for the *'three-element viscoelastic material model'* obtained from a variable β_r parameter and constant E_{1r}, E_{2r} and τ parameters.

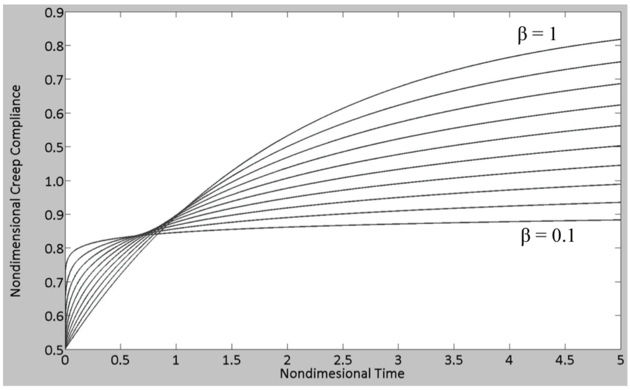

Figure 3. Multiple creep curves for the *'three-element viscoelastic material model'* obtained from a variable β_c parameter and constant E_{1c}, E_{2c} and h parameters.

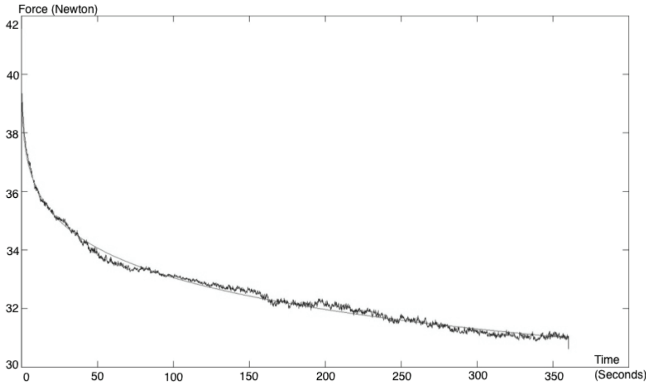

Figure 4. Force relaxation test curve of a specimen (dark line) fitted on the best-matched curve of the *'three-element viscoelastic material model'* (gray line) (by using Matlab®).

was calculated by the formula presented in Equation 5 to evaluate the quality of model fitting;

$$LSE = \sqrt{\frac{\sum_{i=1}^{n}\left[\sigma_{measured}(i) - \sigma_{model}(i)\right]^2}{\sum_{i=1}^{n}\sigma_{measured}(i)^2}} x100 \quad (5)$$

All LSE values calculated were found to be below 1% in all tests. After obtaining the best-fitted curve for each specimen, relaxation data was regressed to determine four relaxation test parameters (E_{1r}, E_{2r}, τ and β_r) and creep data was regressed to determine four creep test parameters (E_{1c}, E_{2c}, η and β_c).

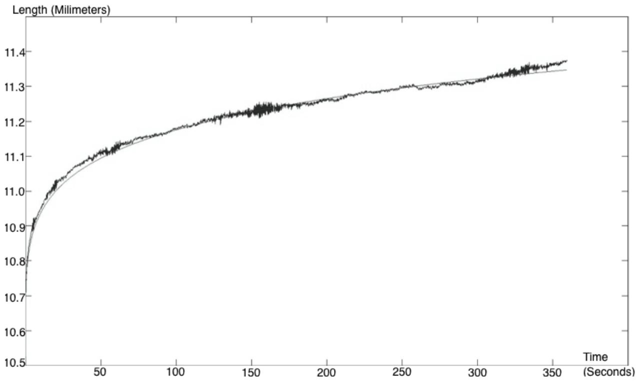

Figure 5. Creep test curve of a specimen (dark line) fitted on the best-matched curve of the *'three-element viscoelastic material model'* (gray line) (by using Matlab®).

Statistical Analysis

Data analysis was performed by SPSS 15.0 software package. Numerical variables were evaluated for normality of data distribution by using Kolmogorov-Smirnov test. Descriptive statistics were expressed as mean ± standard deviation or median (min-max) according to the assumption of normal distribution. The analysis of variance (ANOVA) was used to determine whether significant difference were present between the groups with normal variable distribution. Comparisons between groups in non-normal quantitative variables were evaluated by Kruskal Wallis test. A $p<0.05$ was indicated for statistical significance.

Results

Statistical analysis of the parameters revealed that a significant difference between groups was obtained in two parameters of force relaxation tests. Mean β_r in the control group (0.405 –/+ 0.089) was significantly higher than that in the denervation group (0.301 –/+ 0.071) ($p = 0.042$). In other words, denervated skeletal muscles demonstrated a more solid material behavior in force relaxation tests, while healthy skeletal muscles had a more fluid behavior. Secondly, healthy skeletal muscles achieved a steady state (τ) significantly faster than the denervated ones (83 to 622 seconds, respectively), in relaxation ($p = 0.017$). On the other hand, there was no significant difference between groups for the parameters representing the elastic stiffness (E_{1r} and E_{2r}) of muscle, in relaxation tests. Also there was no statistically significant difference between groups for any parameters of creep tests (E_{1c}, E_{2c}, η and β_c). Results have been summarized in Tables 1 and 2.

Discussion

Like most biological tissues, skeletal muscle has viscoelastic material properties as time and strain

Table 1. Variance analysis results for the parameters demonstrating normal distribution were summarized as mean (–/+ standart deviation). E_{1r}, E_{2r}; parameters of elastic stiffness in force relaxation. β_r; solid-fluid behavior in force relaxation. E_{2c}; parameter of elastic stiffness in creep.

Parameter	Control Group Mean (std.dev.)	Denervation Group Mean (std.dev.)	p value
E_{1r} (N/mm)	41.175 (–/+ 1.122)	41.849 (–/+ 0.615)	0.086
E_{2r} (N/mm)	56.477 (–/+ 18.342)	29.982 (–/+ 22.234)	0.075
β_r	0.405 (–/+ 0.089)	0.301 (–/+ 0.071)	**0.042***
E_{2c} (mm/N)	0.238 (–/+ 0.200)	0.405 (–/+ 0.185)	0.081

Table 2. Non-parametric test results for non-normal quantitative variables were summarized as median (min-max). η; time parameter for creep. E_{1c}; elastic stiffness parameter in creep. τ; time parameter for relaxation. β_c; solid-fluid behavior in creep.

Parameter	Control Group Median (min-max)	Denervation Group Median (min-max)	p value
τ (seconds)	83.298 (33.718–243.222)	622.404 (47.574–2267)	**0.017***
E_{1c} (mm/N)	0.109 (0.08–0.98)	0.09 (0.045–0.117)	0.058
η (seconds)	3527.3405 (60.623–18276)	8547 (345.6–45233)	0.265
β_c	0.318 (0.288–0.775)	0.289 (0.237–0.561)	0.384

dependent responses to tensile loads [9,10]. Several studies have demonstrated that, skeletal muscle exhibits 'viscous behavior' depending on the rate of applied stretch and 'elastic behavior' depending on the load of the applied stretch [11]. The structural components composing viscoelastic properties are stable cross-links between actin and myosin, non-contractile intra- and extra-cellular components of the cytoskeleton (such as Z-discs, T-tubules, titin, desmin) and surrounding connective tissue elements (endo-, epi-, perimysium) [2,12]. Biomechanical studies have demonstrated that tendons have relatively constant length and can be neglected in the passive length-tension responses in physiologic loads [13,14]. However, inter-relationships of these structural components and how they contribute to passive extensibility characteristics remains unclear.

'Passive length-tension curve' can give information about changes in the passive forces, length extensibility and passive elastic stiffness of a single muscle after morphologic and histologic alterations. Variations in the position and the steepness of the curve indicate the changes in passive mechanical properties. For instance, a displacement in the initial and end points of the curve indicates the changes in passive extensibility. Whereas, shallowness or steepness of the length-tension curve indicate changes in passive elastic stiffness [15]. In this study, mathematical modelling of the passive mechanical properties allows to describe further complex viscoelastic parameters such as the speed of relaxation/creep, elastic stiffness, material behavior and the effects of denervation over them.

Denervation has rapid and prominent changes on skeletal muscle such as decrement in muscle mass and contractile forces [4,5]. After denervation, a rapid loss of sarcomeres up to 35% and shortening of the muscle occurs [16]. At microscopic level, the microvascular architecture deteriorates and collagenous tissue accumulates in the intra and extracellular area [17,18,19]. Previous studies have demonstrated that these histomorphological changes induce some adaptations in length extensibility and passive elastic stiffness of skeletal muscle. For example, denervation causes a decrease in extensibility between initial length and the maximal length [6]. Moreover, the passive length-tension curve becomes steeper, indicating an increase in passive elastic stiffness [7].

In early attempts of modelling passive viscoelastic behaviors, skeletal muscle has been assumed to demonstrate linear elastic and viscous responses [3]. However, further research revealed that both elastic and viscous components of muscle tension have significant nonlinearity, so viscoelastic behavior should be analyzed in the framework of nonlinear mechanics [20]. Fractional calculus applications in the viscoelastic models comes from the idea that, for intermediate materials in between solid and fluid such as biological tissues, there should be a non-integer order derivative relation between stress and strain. By replacing the *'dashpot'* in the linear integer order viscoelastic models with a *'spring pot'*, fractional order viscoelastic models were represented [8]. The *'three element fractional viscoelastic model'* used in this study was firstly described by Caputo and Mainardi.They generalized the integer order derivatives in the standard linear solid model to fractional order [21]. Several biological soft tissue applications of miscellaneous fractional order viscoelastic models have been reported in the literature [22,23,24,25]. These studies indicate that fractional order models are better in representing viscoelastic material behavior of soft biologic tissues rather than standard linear solid models.

In this study, passive mechanical tests on healthy and denervated rabbit quadriceps muscles displayed standard *'stress relaxation'* response under constant strain and *'creep'* response under constant load, as a viscoelastic material. Among eight parameters introduced by mathematical modelling, two parameters for force relaxation tests demonstrated significant difference between control and denervation groups. Firstly, b parameter of denervated muscles displayed more elastic solid behavior when compared with healthy muscles. We consider that histomorphologic adaptations after denervation such as the connective tissue accumulation to intra- and extracellular spaces caused this difference. Secondly, healthy muscles significantly relaxed and reached to the steady state faster than the denervated ones. We introduce this as a novel finding concerning the passive mechanical adaptations of skeletal muscle after denervation. We also propose that it should trigger further research to develop new strategies in the management of chronic denervation.

On the other hand, in this study, creep tests did not demonstrate significant difference between groups for any parameters. In a literature review about passive extensibility of skeletal muscle, Gadjosik stated that creep behavior explains the immediate increase in passive range of motion (ROM) in response to stretching exercises [15]. In light of this comment, we suggest that stretching exercises would cause a similar immediate change in passive ROM on healthy and denervated muscle.

As a limitation of the study, the efficiency of the *'three element fractional viscoelastic model'* in modelling skeletal muscles or any other biological tissue has not been validated before. Despite our observation that this model displayed a good 'curve fitting' with the experimental data with least-squares errors remaining below 1%, the efficiency of this model should be validated with further studies comparing with other linear or nonlinear viscoelastic material models.

There are many advantages of the rabbit femoral nerve-quadriceps muscle model such as the single nerve-muscle innervation relationship, the simplicity of the surgical technique and postoperative care of denervation and muscle isolation procedures, simple and reproducible examination of denervation with the patellar tendon reflex.

In conclusion, denervated skeletal muscles demonstrate more 'near to solid material properties' than healthy ones in relaxation tests. In addition, healthy skeletal muscles relax to a steady state faster than denervated ones. We propose that the alterations of passive mechanical properties caused by any pathological conditions of the skeletal muscle may also be analyzed with viscoleastic models, utilizing fractional calculus.

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