Cole-impedance parameters representing biceps tissue bioimpedance in healthy adults and their alterations following eccentric exercise

Bo Fu, Todd J. Freeborn *

Dept. Electrical and Computer Engineering, The University of Alabama, Tuscaloosa, USA

HIGHLIGHTS

- Tissue bioimpedance from 10 kHz to 100 kHz collected from healthy adults bicep tissues are well represented using the Cole-impedance model, a fractional-order equivalent circuit.
- The Cole-impedance model parameters extracted from pre-eccentric exercise and post-eccentric exercise timepoints (at 72 h and 96 h) show statistically significant decreases of resistances and increases of CPE pseudo-capacitance that align with periods of maximum tissue swelling post-exercise.
- No significant differences in Cole-impedance model parameters immediately post-fatigue compared to pre-fatigue measures.

GRAPHICAL ABSTRACT
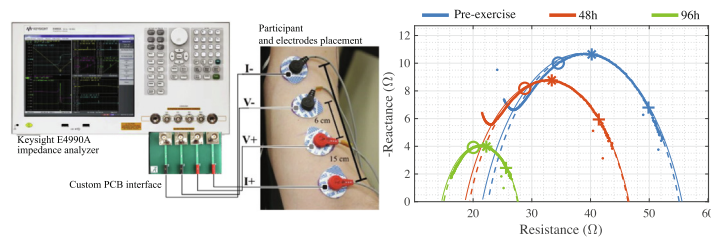
ABSTRACT

The purpose of this study is to identify if participation in an eccentric exercise protocol altered the Cole-impedance model parameters that represent localized bicep tissue bioimpedance. This supports continued efforts to identify which features of tissue bioimpedance may be effective markers to non-invasively identify skeletal muscle damage. Here, the Cole-impedance model parameters that best fit the localized electrical impedance of exercised (using an eccentric stimulus) and unexercised biceps of 6 participants (collected before, immediately after and at 24 h, 48 h, 72 h and 96 h) are determined using a numerical optimization technique. Statistical tests comparing the pre-exercise and post-exercise model parameters report significant decreases in R_∞ and R_1 with significant increases in C at 72 h and 96 h post-exercise for exercised biceps (aligning with noted periods of peak swelling). These changes in R_∞, R_1, and C were not observed in the unexercised biceps. These results support that the C parameter of the Cole-impedance model fit to bioimpedance data may be a suitable marker for identifying skeletal muscle damage.

© 2020 The Authors. Published by Elsevier B.V. on behalf of Cairo University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

The electrical impedance of a biological tissue, referred to as bioimpedance, is a measure of the passive electrical properties of a tissue. These properties are being investigated as a non-invasive technique to characterize the underlying physiological

properties of the measured tissue [1,2]. Tissue bioimpedance is related to the type of tissue, intra/extra cellular fluids, and overall tissue structure [3]. Bioimpedance measurements have been utilized for applications including: body composition determination [4,5], clinical tomography [6], cardiography [7], respiratory monitoring [8], and even food monitoring applications [9]. Bioimpedance is also currently being investigated as a noninvasive technique to characterize skeletal muscle tissue with a particular focus on assessing neuromuscular disorders [10,11,2]. Beyond assessing neuromuscular disorders, bioimpedance is being investigated to monitor tissue changes that result from the exercise and fatigue of skeletal muscle, with decreases in impedance magnitude reported after resistance training protocols [12–16], with further results reporting decreased in impedance magnitude for acute muscle injury [17,18].

Non-invasive methods to monitor skeletal muscle for markers of fatigue and injury could support improved management of activity and workloads for populations severely impacted by injury. Groups such as nurses, construction workers, athletes, and service members are all populations that experience declines in task performance resulting from fatigue which can reduce productivity, safety, and health. In fact, nursing is one of the professions with the highest rates of workplace injury [19]. Recently Thompson et al. reported that isometric strength decreased up to 19.2% in nurses after completing 12-h work shifts in a four-day period, supporting that functional performance declines occur as a result of typical nursing activities (bending, twisting, extreme flexion, and heavy lifting) [19]. While the study by Thompson et al. does not link fatigue and injury, injury risks may increase as individual lifting technique alters to compensate for fatigue-related performance declines. Therefore, the monitoring skeletal muscle fatigue in nursing professionals may provide opportunities to manage individual workloads and tasks to reduce fatigue and injury rates within this population. Other professionals with significant rates of skeletal muscle injury could similarly benefit from monitoring methods to optimize workload, fatigue, and risks of injury.

This provides the motivation for investigating the changes in tissue bioimpedance resulting from fatiguing activity, to understand if this technique can be utilized to identify and quantify skeletal muscle fatigue and injury risk. This may provide an alternative lower-cost and lower-resource approach to monitoring skeletal muscle that does not require imaging (e.g. ultrasound or MRI), biopsies, or blood draws.

Bioimpedance analysis approaches

Regardless of the application, when analyzing collected bioimpedance data two approaches are typically adopted: (i) analyzing a single or multiple discrete frequencies [17,12,13,16] or (ii) reducing a multi-frequency impedance spectra to an equivalent circuit representation [20,14,15]. While analyzing discrete frequencies is useful to identify if changes are occurring, it is difficult to link these changes to specific properties of the tissue and its structure. The use of electrical equivalent circuits attempts to connect the impedance measurements to a behavioural or physiologically derived electrical circuit model of the tissue. This approach requires selecting an appropriate circuit model and finding the circuit parameters that best fit the experimental data. For bioimpedance data, a widely utilized equivalent circuit model is known as the Cole-impedance model and was introduced by Kenneth Cole in 1940 [21]. This model has been widely used to model data in biology and biomedicine with a survey of applications provided in [22]. The Cole-impedance model contains 3 components, including 2 resistors (R_∞, R_1) and a constant phase element (CPE). This equivalent circuit model is shown in Fig. 1(a). A CPE is a component with current–voltage characteristics given by:
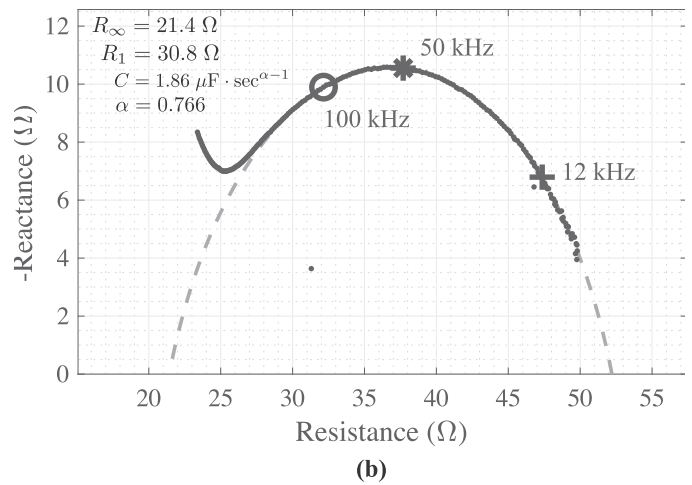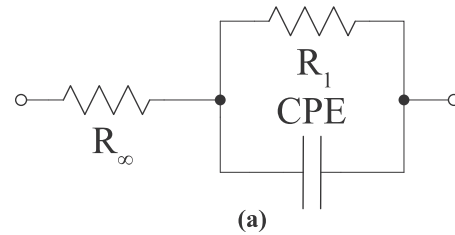

(a)

(b)

Fig. 1. (a) Theoretical Cole-impedance model composed of two resistors (R_∞, R_1) and a constant phase element (CPE) and (b) experimental bioimpedance dataset of bicep tissues (solid) compared to simulated Cole-impedance model impedance (dashed).

$$i(t) = C \frac{d^\alpha v(t)}{dt^\alpha} \tag{1}$$

where $0 < \alpha < 1$ is the order of the derivative. This places a CPE in the domain of fractional-order calculus [22]; the mathematical field concerning integrals and derivatives of non-integer orders. For reference, the Grunwald–Letnikov definition of a fractional derivative of order α is given by:

$$D^\alpha f(x) = \lim_{h \to 0} \sum_{m=0}^{\infty} \frac{(-1)^m}{h^\alpha} \frac{\Gamma(\alpha+1)}{m!\Gamma(\alpha-m+1)} f(x-mh) \tag{2}$$

where $\Gamma(\cdot)$ is the gamma function. Fractional calculus is being investigated for modeling biological phenomena and is often able to represent biological data using models with fewer parameters than integer-order models [22,23].

The electrical impedance of a CPE is $Z_{CPE} = 1/C(j\omega)^\alpha$, where C and α are the pseudo-capacitance and fractional-order. With the fractional-order fixed in the interval $0 < \alpha < 1$, the CPE has electrical characteristics that place it between a resistor ($\alpha = 0$) and an ideal capacitor ($\alpha = 1$). In this fixed interval, a CPE is often as referred to as a fractional-order capacitor which is why it is being represented using a capacitor schematic representation in Fig. 1(a). The units of a CPE/ fractional-order capacitor proposed by Westerlund and Ekstam are $F \cdot sec^{\alpha-1}$ [24], though often CPE units are presented in Farads. Therefore, the electrical impedance of the Cole-impedance model in Fig. 1 is expressed as:

$$Z = R_\infty + \frac{R_1}{1 + (j\omega)^\alpha R_1 C} \tag{3}$$

The Cole-impedance model has been applied to characterize the electrical impedance of biological tissues [22,23], showing a good fit with the frequency dependent tissue impedance. A sample of a bioimpedance dataset collected from the bicep tissue of a study

participant is given in Fig. 1(b) as a solid line. The simulation of (3) using the parameters $R_\infty = 21.4\ \Omega, R_1 = 30.8\ \Omega, C = 1.86\ \mu\text{F}\cdot\text{sec}^{\alpha-1}$, and $\alpha = 0.766$ are also given as a dashed line to highlight that the Cole-impedance model is able to represent this impedance dataset. The simulated (dashed) impedance fits the dataset well until the experimental (solid) displays the hook artifact (an increase in reactance at high frequency). This hook artifact is attributed to capacitive parasitics in the experimental test setup and are not representative of the tissue under study [25].

The Cole-impedance model has been used to represent the 10–100 kHz bioimpedance of the biceps tissues of healthy adults before and after participation in an isotonic exercise protocol [14,15]; where Freeborn and Fu reported statistically significant decreases in the resistance parameters of the Cole-impedance model (R_∞, R_1) comparing the pre-exercise and post-exercise measures [15]. The resistance of a tissue is related to the concentration of dissolved ions in the biological medium [26], therefore R_∞ and R_1 of the Cole-impedance model are expected to be associated with the intracellular and extracellular fluid in a tissue. This is supported by decreases in R_∞ and R_1 for study participants bicep bioimpedance post exercise [15]. While the resistance parameters showed a decrease, Freeborn and Fu reported no statistically significant differences in the pre/post exercise CPE parameters (C, α) of the study participants bicep tissues [15]. Changes in the CPE parameters are hypothesized to be associated with the cellular membrane properties of the localized tissue. A change in capacitance is dependent on the permittivity of a biological tissue under test [26], as well as the tissue geometry and structure. Muscle damage/injury can induce sarcotubular membrane changes that alter the geometry/structure of a tissue. These alterations from damage are hypothesized to change the CPE parameters of the Cole-impedance model. However, this was not observed in [15] which may indicate that muscle damage or injury did not occur as a result of the specific exercise stimulus, or that the tissue damage has a delay between when the damage occurs and when markers of the damage (e.g. inflammation) can be captured in tissue bioimpedance. Since markers of muscle damage were not collected by Freeborn and Fu no conclusions regarding muscle damage induced from the protocol could be drawn. Additionally, only data immediately post-exercise was analyzed, preventing any conclusions regarding further bioimepdance changes in the tissues with time. Further research is needed to explore if the CPE parameters of Cole-impedance model representations of skeletal muscle are altered in response to exercise stimuli expected to induce reversible muscle damage. If the CPE is sensitive to localized tissue damage, this approach may be an effective technique for non-invasive skeletal muscle monitoring. Exploring this aim requires a higher stimulus exercise protocol than previous studies to induce (reversible) tissue damage and collecting measurements at further time-points post exercise. Both of which were accomplished in a follow-up study by Freeborn et al. [16].

This provides the motivation for this study, to expand on the analyses of bicep tissue bioimpedance collected by Freeborn et al. in [16] that only analyzed discrete frequency impedance changes (at 10 kHz, 50 kHz, and 100 kHz) in participants for 96 h following an eccentric exercise protocol to answer the research question: Does participation in an eccentric exercise protocol alter the CPE parameters of the Cole-impedance model that represent the measured tissue bioimpedance?

To explore this research question, the Cole-impedance model parameters that represent the localized biceps tissue bioimpedance of 6 participants that participated in an eccentric exercise protocol of the elbow flexors of one arm were determined and compared against the parameters that represent the tissue bioimpedance collected from their unexercised arms. This data

was collected at 6 different timepoints (pre, immediately post, 24 h, 48 h, 72 h, and 96 h post) to track the impedance changes over time that resulted from participation in the eccentric exercise protocol. This manuscript is organized as follows: Section "Introduction" provides background on bioimpedance measurements and their applications; Section "Materials and methods" details subject recruitment, exercise protocols, data measurement procedures, and optimization procedures for Cole model parameter determined; Section "Results" details the Cole model parameters determined for the study participants; Section "Discussion" discusses the implications of this study and comparisons to other works in this field; Section "Conclusion" concludes and summarizes this study.

Materials and methods

The bioimpedance data from the participants that was analyzed for this research was collected and reported initially by Freeborn et al. in [16]. While the complete details of the study participants and exercise protocol are available in [16], they are also summarized here for readers.

Subjects recruitment

A group of six healthy young adults (20–25 years old) were recruited to complete an eccentric exercise protocol using an elbow flexor activity under the supervision of the study personnel. All subjects were recruited from the University of Alabama in Tuscaloosa, Alabama, USA. No participants reported muscle or joint problems or recent adverse reactions to exercise. Prior to their participation in the eccentric exercise protocol, each participant provided their written informed consent. All of the study procedures were approved by the University of Alabama Institutional Review Board (16-OR-212).

Exercise protocol

An eccentric exercise protocol was utilized for the exercise stimulus because eccentric load resistance training has been reported to induce greater muscle damage compared to concentric and isotonic exercise [27,28]. Each participant executed 50 eccentric bicep curl repetitions using a dumbbell at 90% of their previously assessed 1-RM concentric weight. This is the maximum weight that each participant was able to successfully lift for one single repetition of this exercise. The 50 eccentric contractions were executed in 2 sets of 25, with a two-minute rest between sets. Each repetition required 2-3 seconds to be performed with the participant slowly lowering the dumbbell (held in the palm of their hand) from shoulder height until full extension of the elbow with the dumbbell coming to rest near waist height. To isolate the exercise to only this eccentric component the study personnel lifted the dumbbell back to the shoulder height to initiate the next repetition. The choice of left or right arm for the exercise protocol was self-selected by the participant at the beginning of the study. This self-selection was done so participants could limit the impact of muscle soreness post-protocol on their daily living.

Bicep measurements

The biceps tissue bioimpedance was measured using a tetrapolar configuration of Ag/AgCl electrodes placed on the bicep skin surface, as shown in Fig. 2. The two current injection electrodes (I+, I−) were fixed at a distance of 15 cm with the voltage sensing electrodes (V+, V−) fixed 6 cm apart. The location of the electrodes after initial placement was marked on the skin by a medical
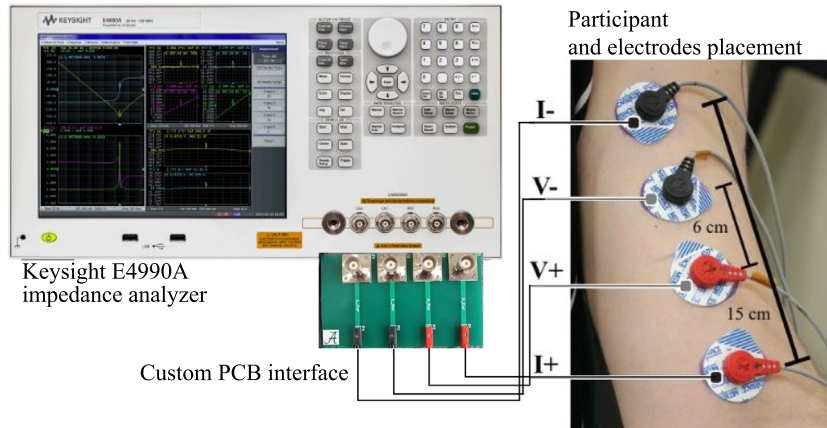
Fig. 2. Experimental setup for bioimpedance measurement using impedance analyzer Keysight E4990A, custom PCB interface and Ag/AgCl surface electrodes placement on participant's biceps brachii tissue.

marker to ensure similar placements in each of the post-exercise measurements. Measurements were collected from both arms before the exercise, immediately after the exercise, and at 24 h, 48 h, 72 h and 96 h post exercise.

The positions of electrodes do impact the measured electrical impedance of the tissue, since the position of current injection electrodes influences the potential region that current will flow through the tissue and the position of voltage sensing electrodes influences the region of tissue that will be measured. This can result in larger resistance/reactance values for electrodes with greater separation distances, yet there is no widely adopted process to standardize electrode placement for localized bioimpedance measurements; though recent guidelines have been proposed by Sanchez et al. [29]. Without standardization, care must be taken when comparing measurements from different studies with different electrode configurations. A fixed distance electrode configuration was utilized for data collection to limit electrode spacing as a confounding variable in the analysis.

The surface electrodes were interfaced to a Keysight E4990A impedance analyzer using a custom PCB and cable sets, also detailed in Fig. 2. The impedance analyzer collected one sweep of measurements from 5 kHz to 1 MHz at 201 logarithmically spaced frequencies. Only one sweep was performed because Keysight E4990A was configured to collect measurements at its highest precision setting (MEAS = 5), which required approximately 60 s to collect a single sweep. Collecting multiple sweeps would require participants to remain motionless for a longer period of time (increasing the risk of movement or contraction artifacts being captured in the data) and increasing the chance the measurements separated by minutes of time may be capturing different physiological states of the tissue. It was for these reasons that only a single sweep was utilized here. While measurements from 5 kHz to 1 MHz were collected, only data from 12 kHz to 100 kHz were used for fitting based on previous reports that this frequency range was least impacted by large residual impedances present in bioimpedance measurements [30]. Further details of the instrument setup for the data collection from this group of participants are provided in [16]. It should be noted that the measured impedances across multiple days have differences in the test configuration resulting from different cable positions and electrodes used on each day. Previous studies by Fu and Freeborn showed that the variability of the test setup utilized in this study was < 1.15% and < 2.6% for resistance and reactance measurements (in the study frequency band) due to changes in cable positioning [13]; which was less than the bioimpedance changes reported for tissue measurements after exercise.

In addition to the tissue bioimpedance, the circumference of the biceps (measured at the centerpoint between the voltage sensing electrodes) of each participant was measured with a cloth tape measure at each time point immediately prior to the bioimpedance measurements. These measurements were collected to assess the amount of swelling that occurred in the tissue as a result of the eccentric protocol.

Cole-impedance model fitting

For each collected bioimpedance measurement, the Cole-impedance model parameters (R_∞, R_1, C and α) were determined by applying a numerical optimizing fitting to identify the parameters that minimized the squared difference between both real and imaginary components of the experimental data and model. The objective function for this numerical optimization is given as:

$$\min_{\boldsymbol{x}} f_0(\boldsymbol{x}) = \sum_{k=1}^{n} \left[(\Re\{Z_k(\boldsymbol{x}) - y_k\})^2 + (\Im\{Z_k(\boldsymbol{x}) - y_k\})^2 \right]$$
$$\text{s.t.} \boldsymbol{x} > \boldsymbol{0}$$
(4)

where $f_0(x)$ is the objective function, n is the number of discrete frequencies used for fitting (81 in this study), $R\{\cdot\}$ and $I\{\cdot\}$ are operators for the real and imaginary parts of a complex number, y_k is the collected bioimpedance at $k-th$ frequency and $Z_k(x)$ is the impedance of Cole-impedance model with x (the vector of the model parameters R_∞, R_1, C, α). Solving (4) requires an iterative process, searching for the model parameters that ideally reduce the objective function to zero. The numerical optimization process applied for solving the problem stated in (4) utilized the gradient descent method. The selection of the gradient descent method is based on its application in previous bioimpedance studies [15] and wide accessibility using MATLAB. Admittedly, there are other available optimization algorithms available such as Chaotic Flower Pollination [31], Grew Wolf optimizer [31], Moth-Flame optimizer [32], Bacterial Foraging optimization [32], and Particle Swarm Optimization [33]. The ease of accessibility and usability of implementing the GD technique makes it an appropriate choice for this work. Future work should further explore the use of different optimization algorithms applied to experimental bioimpednace datasets to compare accuracy and resources required for each method.

The MATLAB function `fmincon` was applied as the GD method to every bioimpedance dataset. For this implementation, the solver was applied 50 times, with a randomly generated initial condition used for each iteration. This approach was taken to minimize the likelihood of returning a local minima as the global solution. The

global solution was determined as the one with lowest objective function value. This approach has been previously utilized in [15] yielding results with good agreement with experimental bicep tissue bioimpedance. For reference, the default MATLAB settings were used for the exit criteria for this algorithm in terms of the maximum number of functions iterations and minimum function change. A constrained search was utilized for this optimization procedure. Specifically, the lower bounds for R_∞, R_1, C and α were $1\,\Omega, 1\,\Omega\ 1\ nF \cdot sec^{\alpha-1}$, and 0.5, respectively with upper bounds 50 $\Omega, 50\,\Omega, 100\,\mu F \cdot sec^{\alpha-1}$ and 1. These boundaries have been utilized for previous approaches applying optimization routines for fitting bioimpedance data to electrical equivalent circuits [25].

Results

A total of 72 impedance spectra were analyzed in this study to determine the Cole-impedance parameters that best fit these datasets. These 72 datasets represent the exercised and unexercised bicep tissue impedance of 6 participants measured at 6 different time points (pre-exercise, post-exercise, 24 h, 48 h, 72 h and 96 h hours post-exercise). The complete set of Cole-impedance parameters determined from these impedance datasets are given in Tables 1 and 2 for the exercised and unexercised biceps, respectively.

To highlight the fit of simulations using the Cole-impedance parameters from Tables 1 and 2, both measurements and simulations using the Cole-impedance parameters for 3 timepoints (Pre, 48 h, 96 h) are given in Figs. 3 and 4 for exercised and unexercised biceps, respectively. Notice that all of the presented datasets exhibit the semi-circle arc often associated with a Cole-impedance model spectrum [22]. It should be noted only data from 12 kHz to 100 kHz was used in the fitting. These lower and upper frequency limits are indicated with the plus ('+') and circle ('o') symbols, respectively, on each individual dataset. While experimental bioimpedance data beyond this range was collected, they were not used in the fitting because impedance within the frequency range of 10 kHz and 100 kHz have been identified as the most accurate for the Keysight E4990A during bioimpedance measurements with a large residual impedance [30]. This also limited the effect of high-frequency hook artifact (which the Cole-impedance model cannot fit), observed in the datasets from having a significant influence on the fitting procedures. This hook artifact is an increasing reactance at high frequency, which is attributed to parasitic capacitances present in the measurement configuration and not the tissue under study [25].

From visual inspection, the simulations using (3) with the Cole-impedance model parameters from Tables 1 and 2 show very good agreement with the experimental data in the fitting band (12–100 kHz). The simulations using extracted Cole-impedance param-

Table 1
Cole-impedance parameters from exercised biceps of study participants.

Time point	R_∞ (Ω)	R_1 (Ω)	C $(\mu F \cdot sec^{\alpha-1})$	α $(a.u.)$
Participant 1				
Pre	22.5	32.6	2.32	0.740
Post	20.1	29.0	2.92	0.731
24 h	21.3	29.9	3.37	0.726
48 h	19.4	27.0	3.35	0.731
72 h	17.4	23.4	5.41	0.705
96 h	15.0	12.6	6.44	0.727
Participant 2				
Pre	25.2	35.7	2.11	0.711
Post	26.3	29.9	1.84	0.745
24 h	26.9	32.4	1.93	0.742
48 h	24.7	32.2	2.47	0.717
72 h	15.4	17.9	6.20	0.649
96 h	14.7	12.6	9.31	0.652
Participant 3				
Pre	39.0	42.9	1.62	0.731
Post	23.6	40.2	3.18	0.666
24 h	27.5	37.4	2.20	0.717
48 h	26.4	34.6	2.11	0.721
72 h	29.2	32.0	2.31	0.727
96 h	23.4	21.3	3.20	0.715
Participant 4				
Pre	23.1	38.3	2.46	0.711
Post	20.9	31.7	2.50	0.725
24 h	21.9	29.7	2.31	0.731
48 h	22.8	24.6	3.42	0.720
72 h	15.1	14.7	13.9	0.631
96 h	13.4	14.2	15.0	0.621
Participant 5				
Pre	24.1	35.4	2.30	0.722
Post	23.5	32.5	2.82	0.712
24 h	24.5	35.9	2.36	0.717
48 h	24.2	36.1	2.14	0.725
72 h	20.2	34.2	2.70	0.703
96 h	23.9	39.1	2.87	0.691
Participant 6				
Pre	25.5	37.6	2.77	0.712
Post	23.0	34.7	3.35	0.709
24 h	25.2	34.5	3.22	0.708
48 h	20.1	22.2	13.0	0.635
72 h	15.8	11.0	6.59	0.721
96 h	18.1	16.5	4.47	0.709

Table 2
Cole-impedance parameters from unexercised biceps of study participants.

| Time point	R_∞ (Ω)	R_1 (Ω)	C ($\mu F \cdot sec^{\alpha-1}$)	α (a.u.)
Participant 1				
Pre	20.0	30.2	2.71	0.736
Post	19.3	30.0	2.59	0.737
24 h	21.5	31.8	1.93	0.765
48 h	21.0	31.4	2.17	0.754
72 h	22.8	33.2	2.27	0.746
96 h	21.4	30.8	1.86	0.766
Participant 2				
Pre	24.4	32.4	1.87	0.730
Post	25.6	28.0	1.14	0.781
24 h	24.1	34.1	1.96	0.738
48 h	22.9	35.5	2.25	0.710
72 h	23.6	31.1	2.50	0.720
96 h	24.9	35.1	2.12	0.715
Participant 3				
Pre	30.2	44.9	2.67	0.680
Post	34.1	40.6	1.91	0.723
24 h	29.6	38.1	2.51	0.702
48 h	28.3	43.2	2.04	0.710
72 h	28.8	34.6	2.68	0.706
96 h	30.6	38.1	2.77	0.694
Participant 4				
Pre	22.4	34.9	2.33	0.730
Post	23.0	37.5	2.27	0.722
24 h	22.8	35.9	2.27	0.719
48 h	24.2	38.3	2.36	0.721
72 h	21.1	38.2	2.87	0.700
96 h	21.5	39.9	3.45	0.682
Participant 5				
Pre	26.8	33.6	2.30	0.721
Post	24.8	33.7	3.16	0.698
24 h	24.5	33.6	2.83	0.704
48 h	23.4	30.8	2.32	0.723
72 h	25.1	34.2	3.38	0.687
96 h	23.3	34.8	2.87	0.695
Participant 6				
Pre	28.8	40.7	3.20	0.628
Post	29.2	37.7	2.59	0.707
24 h	25.8	36.9	3.40	0.684
48 h	29.4	36.7	2.09	0.734
72 h	26.8	34.4	3.24	0.691
96 h	27.9	38.3	2.51	0.704
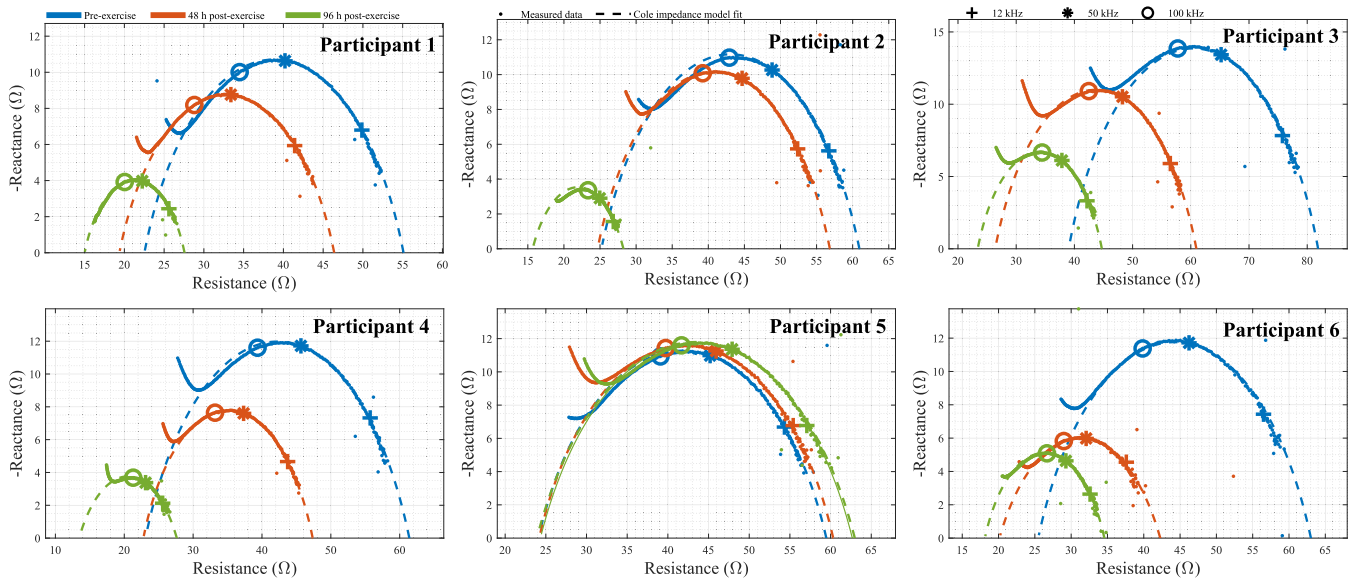
Fig. 3. Sample impedance measurements and simulations (using estimated Cole-impedance parameters from 1 mHz to 100 MHz) for all 6 participants' exercised biceps at 3 timepoints: Pre-exercise (blue), 48 h post-exercise (red), 96 h post-exercise (green). Measured impedance at 12 kHz, 50 kHz and 100 kHz are indicated with ('+'), ('∗') and ('o') signs respectively.

Fig. 4. Sample impedance measurements and simulations (using estimated Cole-impedance parameters from 1 mHz to 100 MHz) for all 6 participants' unexercised biceps at 3 timepoints: Pre-exercise (blue), 48 h post-exercise (red), 96 h post-exercise (green). Measured impedance at 12 kHz, 50 kHz and 100 kHz are indicated with ('+'), ('*') and ('o') signs respectively.
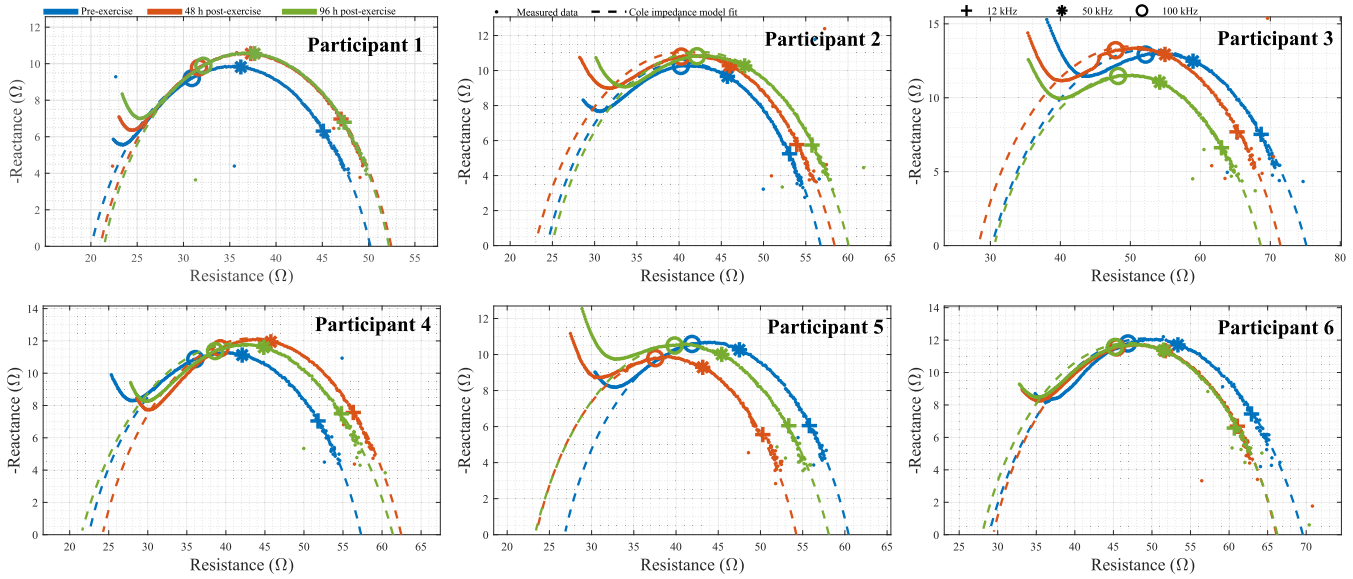
eters in Fig. 3 and 4 are given as dashed lines. Note though, the simulations use an extended frequency band (1 mHz to 100 MHz) to highlight that theoretical low and high-frequency resistance behavior of the model with these parameters. This further validates that the Cole-impedance model, a fractional-order impedance model, can be used to fit collected bioimpedance datasets. From Tables 1 and 2 the fractional-order is within the range from $0.6 < \alpha < 0.76$; far from the ideal case of a capacitor ($\alpha = 1$) for the CPE. Qualitative differences between the experimental datasets, which were reported previously in [16], can be observed in the exercised datasets of Fig. 3 as decreases in both resistance and reactance for most participants (with the exception of Participant 5, which is attributed to this participants history of training and is discussed in [16]). These differences visually manifest as decreases in the arc magnitude and a shift (left) to lower resistances. These same differences are not observed in the unexercised data in Fig. 4.

To compare the extracted parameters of the exercised and unexercised arms and their differences across the different timepoints, the parameters are reduced to their group medians which are detailed in Table 3. From the groups medians, there is a general trend of decreasing R_∞ and R_1 at increasing time-points

post-exercise and increasing C (also at increasing time-points) for the exercised biceps group. Specifically, there are differences of $-31.3\%, -58.2\%, 136\%$, and -2.8% for R_∞, R_1, C, and α, respectively, between the pre and 96 h measurements. These same trends are not observed for the non-exercised biceps group. For comparison, the median differences are $-5.9\%, 6.7\%, 5.6\%$, and -4.1% for R_∞, R_1, C, and α, respectively, between the pre and 96 h measurements.

Further comparison of the parameters at each time-point against the pre-exercise values was conducted using statistical testing. Specifically, pairwise comparisons of each parameter fit using data at post-exercise and 24 h, 48 h, 72 h and 96 h compared to the pre-exercise values were conducted using Friedman tests. The Friedman test is a non-parametric test selected because parameter datasets violated normality, tested using the Shapiro–Wilk test. For tests that showed statistical significant, pair-wise comparisons were made with Bonferroni corrections applied. The level of statistical significant was set at the $p < 0.05$ level. In Table 3, any statistically significant change of a post-exercise parameter compared against pre-exercise values are marked with an asterisks (∗). Statistical tests results of the exercised parameters show statistically significant decreases of R_∞, R_1 and statistically

Table 3
Medians of Cole-impedance parameters from exercised and non-exercised arms of study participants.

Time point	R_∞ (Ω)	R_1 (Ω)	C ($\mu F \cdot sec^{\alpha-1}$)	α ($a.u.$)
Exercised				
Pre	24.6	36.6	2.31	0.72
Post	23.2	32.1	2.87	0.72
24 h	24.8	33.4	2.33	0.72
48 h	23.5	29.6	2.91	0.72
72 h	16.6*	20.6*	5.80*	0.70
96 h	16.9*	15.3*	5.46*	0.70
Unexercised				
Pre	25.6	34.3	2.50	0.73
Post	25.2	35.6	2.43	0.72
24 h	24.3	35.0	2.39	0.71
48 h	23.8	36.1	2.21	0.72
72 h	24.4	34.3	2.77	0.70
96 h	24.1	36.6	2.64	0.70

Note: Significant change ($p < 0.05$) compared to pre-exercise value is indicated with (∗).

significant increase of C at 72 and 96 h after exercise. Note, there are no statistically significant differences between any of the comparisons for the unexercised Cole-impedance parameters.

Discussion

Cole-impedance parameter alterations

The increases in C reported for the exercised arm tissue bioimpedance at 72 h and 96 h after the eccentric protocol, which were not observed in the unexercised arms are the significant finding in this analysis. This supports that monitoring changes in C when skeletal muscle bioimpedance is represented using the Cole-impedance model may be an effective avenue to identify tissue damage. Previously, Freeborn and Fu reported only decreases in R_∞ and R_1 of bicep bioimpedance immediately after participation in an isotonic protocol [15]; with no statistically significant changes of C. With the hypothesis that the CPE properties (C, α) are associated with the cellular membrane, the lack of change in [15] could indicate that there was no damage to the cellular membrane from the isotonic exercise protocol [15].

In contrast to the changes in tissues after an isotonic protocol [15], the results of this analysis report statistically significant increases in C in addition to decreases of R_∞ and R_1. Further, these differences occur at later timepoints (72 h, 96 h) compared to immediately post-exercise in [15]. This is attributed to the eccentric exercise protocols utilized as the stimulus in data analyzed in this work. The use of the high-repetition, high-load eccentric activity as the exercise stimulus was expected to result in more significant localized skeletal muscle damage. Eccentric training often results in delayed-onset muscle soreness (DOMS) within $1 - 3$ days [34–36,27] as a result of swelling and inflammation in the localized tissue from the exercise induced muscle damage. This delay from exercise to swelling/soreness is expected to also be the reason for the delay in the changes in the Cole-impedance parameters.

While markers of muscle damage were not collected in this study (which is a limitation of the study), the circumference of the exercised and unexercised biceps were measured at each time-point to quantify the swelling induced by the protocol as an indirect measure of damage. As noted in [16], the study participants analyzed here had increased bicep circumferences at 48 h, 72 h, and 96 h compared to the pre-exercise measures; without circumference changes reported for the unexercised biceps. This swelling supports that localized inflammation occurred post-exercise, induced by the eccentric exercise. The R_∞, R_1, and C parameters for the exercised biceps determined in this analysis show statistically significant differences at the 72 h and 96 h timepoints; overlapping with the period of swelling as determined by the bicep circumference measurements [16]. R_∞ and R_1 are attributed to extra and intracellular fluids [37], with decreases in these parameters indicating swelling/fluid increases. But the change in C could indicate changes in the underlying cellular membranes from damage.

The parameter C indicates the ability of tissue to be polarized and is associated with tissue permittivity [26] and muscle cellular/subcellular structure. Changes of muscle structure, including myofibrillar disturbances, Z-line streaming and disturbed arrangement of filaments at the A-band, have been noted to occur after eccentric exercise [38]. Hence, the increase in C observed in the exercised biceps of participants may be a result of muscle damage induced by the eccentric exercise protocol. Statistically significant changes in the R_∞, R_1, and C for the unexercised biceps were not observed at any timepoint (compared to the pre-exercise values), supporting that exercised bicep changes were not a result of normal tissue variations associated with daily living.

This work supports using the C parameter as a potential marker of muscle damage when analyzing bioimpedance datasets of skeletal muscle and may be more strongly associated with tissue injury/damage than measures of tissue reactance alone. While tissue reactance is expected to be associated with cellular membrane properties, the complex interconnection of materials in a tissue result in impedances that are not uncoupled using only the resistance and reactance (and why equivalent electrical circuit modeling is explored in this work). Freeborn and Fu noted that changes in R_1, associated with fluid increases in a tissue, also alter the reactance of the Cole-impedance [15]. Therefore, changes in discrete frequency reactance may not be a result of tissue damage.

This limitation of discrete impedances combined with the results from this study (increase in C during periods of swelling/inflammation) warrants the continued study of the overall association with the C parameter and muscle damage. However, further work is needed to determine the associations of this Cole-impedance model parameter with gold standard measures of tissue damage (e.g. blood markers or muscle biopsies) and to link this parameter to the actual physiological mechanism of change in the tissue. Also, future studies should monitor for longer periods post-exercise to explore if the C mechanisms return to the pre-exercise values to be used as a marker for recovery as well as overall injury.

An interesting aspect from the analysis of this study is that the CPE order (α) did not have significant differences associated with exercise and was in the same range, 0.6–0.8 across participants in the study. This may indicate that the CPE order of a tissue is associated with the tissue type, related to the material property rather than its geometry [1], and not altered through swelling or changes due to inflammation or muscle damage. Reports by Rigaud et al. of bioimpedance data excised sheep tissues [20] support that the CPE order is linked to the tissue type. Specifically, Rigaud et al. reported CPE orders of 0.71, 0.54, 0.39, and 0.64 for muscle, liver, lung, and spleen samples [20]. The CPE order of skeletal muscle from sheep (0.71) is within the same range of the localized bicep tissue measurements in this study. While the tissue measurements in this study were not from excised tissue, the similar order to the sheep muscle (and significant difference compared to liver and lung tissues) of the biceps tissue (expected to be dominated by skeleton muscle) support that the CPE order may be linked to the tissue type. However, further studies are necessary to investigate this claim.

Of interest for future implementation of the optimization procedures in systems to extract the Cole-impedance parameters from tissue measurements locally on embedded systems (versus the post-processing done on a computer in this work) are the necessary computing resources and execution time. While not investigated in this work, future studies should evaluate the performance of other available numerical optimization algorithms on resource constrained hardware.

Conclusion

The purpose of this study was to identify if participation in an eccentric exercise protocol altered the Cole-impedance model parameters that represent localized bicep tissue bioimpedance. From analysis of tissue bioimpedance, statistically significant decreases of R_∞ and R_1 and significant increases of C were observed in the exercised biceps at 72 h and 96 h post-exercise. These same changes were not observed in the unexercised biceps. This change in C is significant as it is hypothesized to be linked to the cellular membrane properties of the tissue and a potential marker of tissue membrane damage as opposed to tissue fluid. This supports that C

may be an effective marker of skeletal muscle damage which warrants further investigation to determine the association between C to model bioimpedance data collected from tissues and accepted markers of tissue damage.

Compliance with Ethics Requirements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study. All the study procedures were approved by the University of Alabama Institutional Review Board (#16-OR-212).

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.

References

[1] Martinsen OG, Grimnes S. Bioimpedance and bioelectricity basics. Academic Press; 2011.
[2] Sanchez B, Rutkove SB. Electrical impedance myography and its applications in neuromuscular disorders. Neurotherapeutics 2017;14:107–18.
[3] Rutkove SB, Pacheck A, Sanchez B. Sensitivity distribution simulations of surface electrode configurations for electrical impedance myography. Muscle Nerve 2017;56:887–95.
[4] De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. J Appl Physiol 1997;82:1542–58.
[5] Esco MR, Fedewa MV, Freeborn TJ, Moon JR, Wingo JE, Cicone Z, Holmes CJ, Hornikel B, Welborn B. Agreement between supine and standing bioimpedance spectroscopy devices and dual-energy x-ray absorptiometry for body composition determination. Clin Physiol Funct Imag 2019;39:355–61.
[6] Bayford RH. Bioimpedance tomography (electrical impedance tomography). Annu Rev Biomed Eng 2006;8:63–91.
[7] Fellahi J-L, Fischer M-O. Electrical bioimpedance cardiography: an old technology with new hopes for the future. J Cardiothoracic Vasc Anesthesia 2014;28:755–60.
[8] Blanco-Almazan D, Groenendaal W, Catthoor F, Jane R. Wearable bioimpedance measurement for respiratory monitoring during inspiratory loading. IEEE Access 2019;7:89487–96.
[9] Aboalnaga BM, Said LA, Madian AH, Elwakil AS, Radwan AG. Cole bio-impedance model variations in daucus carota sativus under heating and freezing conditions. IEEE Access 2019;7:113254–63.
[10] Rutkove SB. Electrical impedance myography: background, current state, and future directions. Muscle Nerve: Off. J. Am. Assoc. Electrodiagn. Med. 2009;40:936–46.
[11] Rutkove SB, Caress JB, Cartwright MS, Burns TM, Warder J, David WS, Goyal N, Maragakis NJ, Clawson L, Benatar M, et al. Electrical impedance myography as a biomarker to assess als progression. Amyotrophic Lateral Sclerosis 2012;13:439–45.
[12] Li L, Shin H, Li X, Li S, Zhou P. Localized electrical impedance myography of the biceps brachii muscle during different levels of isometric contraction and fatigue. Sensors 2016;16:581. doi: https://doi.org/10.3390/s16040581.
[13] Fu B, Freeborn TJ. Biceps tissue bioimpedance changes from isotonic exercise-induced fatigue at different intensities. Biomed. Phys. Eng. Express 2018;4:025037.
[14] Freeborn TJ., Bohannan GW. Changes of fractional-order model parameters in biceps tissue from fatiguing exercise. In: 2018 IEEE International Symposium on Circuits and Systems (ISCAS). IEEE; 2018. p. 1–5.
[15] Freeborn TJ, Fu B. Fatigue-induced cole electrical impedance model changes of biceps tissue bioimpedance. Fractal Fractional 2018;2:27.
[16] Freeborn TJ, Regard G, Fu B. Localized bicep tissue bioimpedance alterations following eccentric exercise in healthy young adults. IEEE Access 2020;8:23100–9. doi: https://doi.org/10.1109/ACCESS.2020.2970314.
[17] Nescolarde L, Yanguas J, Lukaski H, Alomar X, Rosell-Ferrer J, Rodas G. Localized bioimpedance to assess muscle injury. Physiol Meas 2013;34:237.
[18] Nescolarde L, Yanguas J, Lukaski H, Alomar X, Rosell-Ferrer J, Rodas G. Effects of muscle injury severity on localized bioimpedance measurements. Physiol Meas 2014;36:27.
[19] Thompson B, Stock M, Banuelas B. Effects of accumulating work shifts on performance-based fatigue using multiple strength measurements in day and night shift nurses and aides. Hum Factors 2017;59:346–56. doi: https://doi.org/10.1177/0018720816677814.
[20] Rigaud B, Hamzaoui L, Frikha MR, Chauveau N, Morucci J-P. In vitro tissue characterization and modelling using electrical impedance measurements in the 100 hz - 10 mhz frequency range. Physiol Meas 1995;16:A15–28.
[21] Cole K. Permeability and impermeability of cell membranes for ions. Cold Spring Harbor Symp Quant Biol 1940;8:110–22.
[22] Freeborn TJ. A survey of fractional-order circuit models for biology and biomedicine. IEEE J Emerg Sel Top Circuits Syst 2013;3:416–24.
[23] Ionescu C, Lopes A, Copot D, Machado JT, Bates J. The role of fractional calculus in modeling biological phenomena: A review. Commun Nonlinear Sci Numer Simul 2017;51:141–59.
[24] Westerlund S, Ekstam L. Capacitor theory. IEEE Trans Dielectrics Elec Insulation 1994;1:586–839. doi: https://doi.org/10.1109/94.326654.
[25] Freeborn TJ, Crenshaw T, Critcher S. Hook artifact correction of localized electrical bioimpedance for improved agreement between different device measurements. Biomed Phys Eng Express 2017;4:015016.
[26] Naranjo-Hernández D, Reina-Tosina J, Min M. Fundamentals, recent advances, and future challenges in bioimpedance devices for healthcare applications. J Sensors 2019;2019.
[27] Chen TC, Lin K-Y, Chen H-L, Lin M-J, Nosaka K. Comparison in eccentric exercise-induced muscle damage among four limb muscles. Eur J Appl Physiol 2011;111:211–23.
[28] Lauritzen F, Paulsen G, Raastad T, Bergersen LH, Owe SG. Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. J Appl Physiol 2009;107:1923–34.
[29] Sanchez B, Pacheck A, Rutkove SB. Guidelines to electrode positioning for human and animal electrical impedance myography research. Sci Rep 2016;6:32615.
[30] Fu B, Freeborn TJ. Residual impedance effect on emulated bioimpedance measurements using keysight e4990a precision impedance analyzer. Measurement 2019;134:468–79.
[31] Yousri D, AbdelAty A, Said L, Elwakil MB, S A, Radwan A. Chaotic flower pollination and grey wolf algorithms for parameter extraction of bio-impedance models. Appl Soft Comput 2019;75:750–74.
[32] Yousri D, AbdelAty A, Said L, AboBakr A, Radwan A. Biological inspired optimization algorithms for cole-impedance parameters identification. Int J Electron Commun 2017;78:79–89.
[33] Gholami-Boroujeny S, Bolic M. Extraction of cole parameters from the electrical bioimpedance spectrum using stochastic optimization algorithms. Med Biol Eng Comput 2016;54:643–51.
[34] Brooks G, Fahey T, Baldwin K. Exercise physiology: Human bioenergetics and its applications., Ed. 4, William R. Glass; 2005.
[35] Heiss R, Hotfiel T, Kellermann M, May MS, Wuest W, Janka R, Nagel AM, Uder M, Hammon M. Effect of compression garments on the development of edema and soreness in delayed-onset muscle soreness (doms). J Sports Sci Med 2018;17:392.
[36] Chleboun GS, Howell JN, Conatser RR, Giesey JJ. Relationship between muscle swelling and stiffness after eccentric exercise. Med Sci Sports Exercise 1998;30:529–35.
[37] Sanchez B, Iyer SR, Li J, Kapur K, Xu S, Rutkove SB, Lovering RM. Non-invasive assessment of muscle injury in healthy and dystrophic animals with electrical impedance myography. Muscle Nerve 2017;56:E85–94.
[38] Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. Am J Phys Med Rehabil 2002;81:S52–69.