Whole Muscle and Single Motor Unit Twitch Profiles in a Healthy Adult Cohort Assessed With Phase Contrast Motor Unit MRI (PC-MUMRI)

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Background: Motor units (MUs) control the contraction of muscles and degenerate with age. It is therefore of interest to measure whole muscle and MU twitch profiles in aging skeletal muscle.
Purpose: Apply phase contrast MU MRI (PC-MUMRI) in a cohort of healthy adults to measure whole anterior compartment, individual muscles, and single MU twitch profiles in the calf. Assess the effect of age and sex on contraction and relaxation times.
Study Type: Prospective cross-sectional study.
Subjects: Sixty-one healthy participants (N = 32 male; age 55 ± 16 years [range: 26–82]).
Field Strength/Sequences: 3 T, velocity encoded gradient echo and single shot spin echo pulsed gradient spin echo, echo-planar imaging.
Assessment: Anterior shin compartment (N = 47), individual muscle (tibialis anterior, extensor digitorum longus, peroneus longus; N = 47) and single MU (N = 34) twitch profiles were extracted from the data to calculate contraction and relaxation times.
Statistical Tests: Multivariable linear regression to investigate relationships between age, sex and contraction and relaxation times of the whole anterior compartment. Pearson correlation to investigate relationships between age and contraction and relaxation times of individual muscles and single MUs. A *P* value <0.05 was considered statistically significant.
Results: Age and sex predicted significantly increased contraction and relaxation time for the anterior compartment. Females had significantly longer contraction times than males (females 86 ± 8 msec, males 80 ± 9 msec). Relaxation times were longer, not significant (females 204 ± 36 msec, males 188 ± 34 msec, $P = 0.151$). Contraction and relaxation times of single MUs showed no change with age ($P = 0.462$, $P = 0.534$, respectively).
Date Conclusion: Older participants had significantly longer contraction and relaxation times of the whole anterior compartment compared to younger participants. Females had longer contraction and relaxation times than males, significant for contraction time.
Evidence Level: 2
Technical Efficacy: Stage 1

J. MAGN. RESON. IMAGING 2023.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/jmri.29028

Received Jul 10, 2023, Accepted for publication Sep 13, 2023.

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A motor unit (MU) comprises of the anterior horn cell located in the spinal cord, the myelinated axon and all of the constituent muscle fibers which are innervated by the axon.[1] As an individual ages there is postulated to be a selective denervation of type II (fast twitch) MUs and a process of axonal sprouting and compensatory re-innervation from type I (slow twitch) MUs.[2,3] Although current evidence is supported by animal studies, there is a paucity of data from human studies. However, it has been shown in patients suffering from sarcopenia that there is an impaired ability to re-innervate lost fibers.[4] This process and other functional changes to skeletal muscle result in the generalized loss of strength and mass of muscle associated with age related sarcopenia.[5] Furthermore, reductions in both single muscle fiber velocity,[6] maximal force production and muscle twitch characteristics in response to electrical stimulation have been documented in older people; with older people producing less force and having longer muscle contraction and relaxation times.[7,8]

Currently, whole muscle twitch profiles are measured using surface or needle electrical stimulation in conjunction with a force transducer.[9] This technique, although simple to apply, does not provide information about individual muscle or single MU twitch dynamics. Other more recent methods which have been used to assess single MU and muscle twitch profiles include high density surface electromyography (HDSEMG) which has been used in superficial muscles to assess MU twitch characteristics pre and post marathon[10]; however, HDSEMG struggles to assess deep muscles. Finally, wearable devices such as second harmonic microscopy have been used to image single MU contractions,[11] however this still requires the invasive use of a needle to insert the microscope.

Phase contrast (PC) MRI is a technique which exploits the property of moving ^{1}H nuclei to create image contrast.[12] The PC sequence employs the use of a pair of bi-polar magnetic field gradients to encode the velocity information. In a previous study, Heskamp et al.[13] demonstrated that PC-MRI can be used in conjunction with in scanner electrical stimulation to measure whole muscle and single MU twitch dynamics, by extracting velocity profiles. They also demonstrated that the extracted velocity profile consisted of two components, one representing muscle fiber contraction and the other representing muscle fiber relaxation. Therefore, PC-MRI offers a novel technique to noninvasively study both whole muscle and in particular single MU contractile properties.

The aim of this study was to apply the PC-MRI technique in a large cohort of healthy adults in order to assess how participant age and biological sex affected the contraction and relaxation times extracted from the twitch profiles of the whole anterior compartment, its individual muscles and single MUs. A further aim was to relate the contraction and relaxation time data from the whole anterior compartment to clinical measurements of muscle strength in the form of the participant's maximum voluntary contraction (MVC) during ankle dorsiflexion and hand grip strength.

Materials and Methods

Participants

The study was approved by the Newcastle University Ethics Committee (reference number: 1852/525/2020) and all participants gave written informed consent prior to enrolment.

We recruited men and women aged between 26 and 82 years. Participants were recruited from a larger cohort study investigating sarcopenia across the Lifecourse (MASS_Lifecourse; PI: Professor Avan Sayer, IRAS number 246888[14]). Participants were approached by letter and then, if they expressed interest, were contacted by a member of the study team to arrange a study visit. Participants were included if they could lie flat in the scanner for up to 60 minutes.

Exclusion criteria included: a positive score for probable sarcopenia in the sarcopenia screen, any anticoagulant or antiplatelet medications (except for aspirin for primary prevention of cardiovascular disease which could be suspended), diabetes mellitus, immunosuppressant medication, contraindication to MRI scanning or a clinical history of neuromuscular disease.

Sarcopenia Screening

Participants were screened for probable sarcopenia using the European Working Group on Sarcopenia in Older Persons guidance (EWGSOP2),[5] by measuring their hand grip strength and chair stand time. To exclude probable sarcopenia, patients were required to have a grip strength (>27 kg [male] and >16 kg [female]) and a chair stand time of <15 seconds.

Hand grip strength was measured before the MRI scan on the participants dominant hand using a Jamar Dynamometer (Lafayette Instruments, Lafayette, Indiana, USA). Participants were in a seated position, grip strength was calculated as the maximum over three attempts.[15] Chair stand time was measured as the time taken to complete five full rises from a seated to standing position without the participant using their arms to push up off the chair.

Experimental Set up and MVC Measurement

The left lower leg of each participant was scanned in a 3 T Achieva X MR scanner (Philips Medical Systems, Best, The Netherlands). The left foot was tightly strapped in a custom-built MR compatible isometric force rig containing a binocular beam load cell (capacity 60 kg, resolution 3 g; Elane, China) which was used to measure the MVC of each participant (Fig. 1a). This force rig was connected to a computer in the MR-control room via an optical fiber. The ankle was positioned at an angle of 90–100° and the knee extended. The knee was supported such that the lower leg muscles were not compressed and the participant's leg was fully relaxed.

Isometric MVC of the anterior compartment muscles was measured by using the MR compatible force plate, as the maximum of three maximum dorsiflexion attempts. This was performed before the MR scan commenced. Participants were guided by a force dial projected onto the scanner (Fig. 1a) and asked to perform dorsiflexion to their maximum capacity.

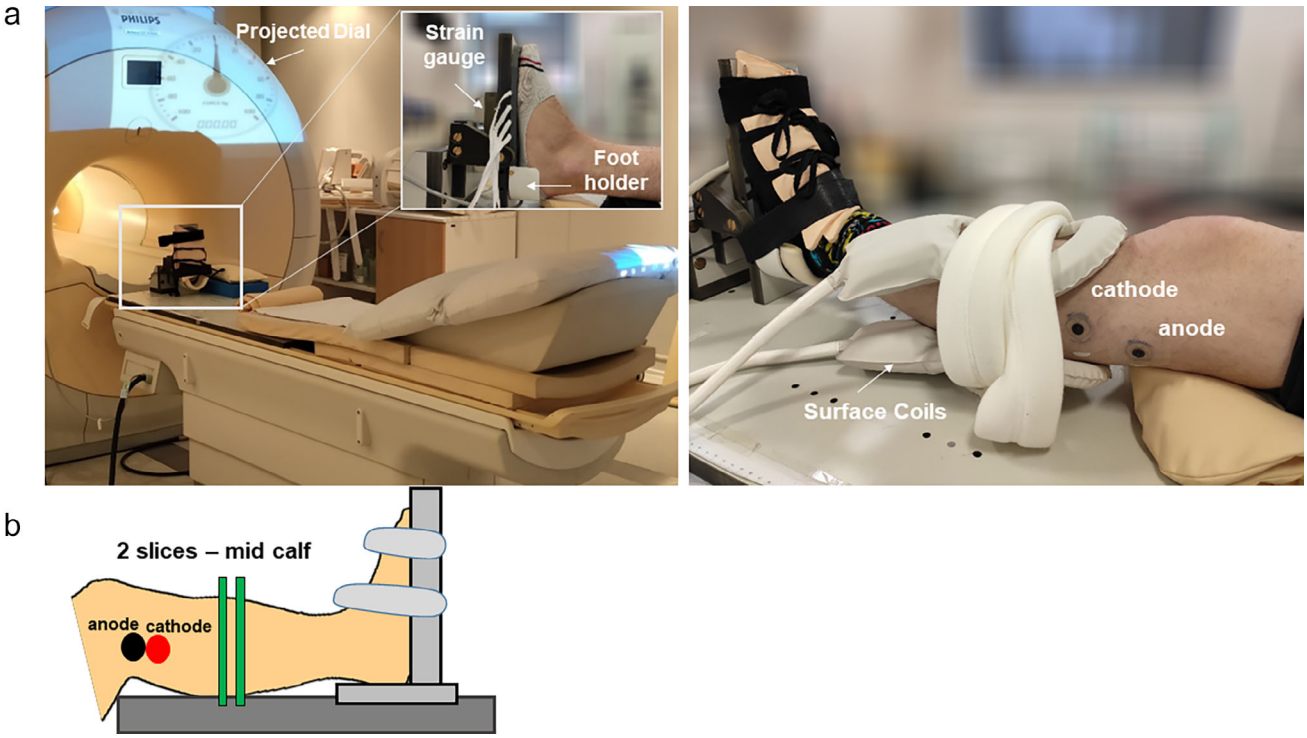


FIGURE 1: (a) Left—MRI scanner showing set-up with the custom-built force plate and also showing the projected dial on the scanner which was used to provide visual feedback to the participant during the maximum voluntary contraction measurements. Inset zoomed image of the foot holder showing position of foot and strain gauge. Right—Shows example placement of the MR imaging coils around the center of the calf and the placement of the cathode and anode electrodes for stimulation. The foot is strapped tightly into the force plate. (b) Schematic showing placement of MR imaging slices for acquiring an image at the thickest part of the calf and placement of cathode (red) and anode (black) with respect to the imaging slices.

Next a pair of 10 cm elliptical flexible surface coils (2 channels) or a torso coil (16 channels) (Philips Medical Systems, Best, The Netherlands) was placed above and below the left lower leg (Fig. 1a). MR images were acquired at the thickest part of the calf (Fig. 1b). Electrical stimulation of the muscles in the anterior compartment was performed using a pair of stimulation electrodes (Cleartrace, ConMed, New York, USA) placed over the left common peroneal nerve (Fig. 1a,b). The inter-electrode distance was 5 cm and the cathode was placed distal (Fig. 1a,b). The stimulation electrodes were connected to a programmable stimulator (DS5, Digitimer, Ft Lauderdale, Florida, USA) via MR compatible coaxial cables with low-pass filters (Minicircuits, New York, USA) at the Faraday cage. A Cambridge Electronic Design 1401 device (Cambridge Electronic Design, CED PLC, UK) was used to generate the stimulator drive pulse and record the data from the force rig and the scanner trigger signal. The control software for the CED 1401 was written in Microsoft Visual Basic by Dr I Schofield, and allowed for a variety of timing procedures.

MR Image Acquisition

MR imaging was performed with two MRI sequences. The first sequence was a diffusion weighted single shot sequence using a pulsed gradient spin echo (PGSE) preparation scheme which is sensitive to the incoherent motion of water which occurs during muscle fiber contraction. The effect of muscle fiber contraction on PGSE images results in brief, localized signal voids within the image.[16] The second was the PC sequence which produces both a magnitude image which shows the anatomical detail of the lower leg muscles and a phase image. The phase image can be used to provide a direct measurement of the velocity of the muscle fiber tissue during muscle fiber contraction and relaxation.[13]

Data were collected during electrical stimulation performed at a frequency of 1 Hz, with a bipolar square pulse (0.3 msec duration). This 1 Hz frequency allowed full relaxation of MUs between stimuli.

The experiment started with a set-up PGSE sequence parameters are given in Table 1. In each acquisition, we acquired approximately 20 repetitions (i.e. images), 1 image every second. The image acquisition was time-locked to the electrical stimulus given. With each repetition, the stimulus current was increased from 0 mA in coarse steps of 0.1–2.0 mA which caused progressive MU activation and appearance of signal voids within the stimulated muscles (Fig. 2). This was performed until a clear level of contrast was observed between the stimulated and non-stimulated muscles (this was defined by eye on the set-up PGSE sequence during the scanning session as the point at which all the stimulated muscles were black compared to the non-stimulated muscles [eg—repetition 13 in Fig. 2]) and the muscle was twitching visibly. This current was defined as I_{muscle}. This was performed by MB (MR Physicist with >7 years' experience) and IS (Research assistant and Neurophysiologist with >40 years' experience).

Twitch Profile Measurement of the Whole Anterior Compartment and Individual Muscles

The next step was to apply a PC sequence with echo planar imaging read-out to capture the temporal shape and velocity of the muscle twitch of the whole anterior compartment and its individual muscles. The PC sequence settings are given in Table 1. We acquired 100 repetitions,

TABLE 1. MRI Sequence Parameters

Sequence Name	Sequence Preparation	Field of View (mm)	Resolution (mm)	Number of Slices	Slice Gap (mm)	Repetition/ Echo Time (msec)	Fat Suppression	Flip Angle (°)	*b* Value (s/mm^2)	Velocity Encoding (VENC) (cm/s)	Encoding Gradient Δ/δ (msec)	Repetitions	Signal Averages	SENSE	Echo Train Length	Acquisition Time (mm:ss)
Set up PGSE	PGSE	160 × 160	1.5 × 1.5 × 8	2	16	1000/36	SPAIR + SSGR + OFS	90/180	20	N/a	16.9/2.2	20	1	1	107	00:20
PC for temporal muscle twitch (whole anterior compartment)	PC Bipolar (4 acquisitions per slice velocity encoding flipped)	160 × 160	1.5 × 1.5 × 8	2	16	500/8.7	SPAIR + OFS	25	N/a	15	2.6/1.3	100	1	1	53	03:21
Coarse Grain PGSE (single motor unit)	PGSE	160 × 160	1.5 × 1.5 × 8	2	16	1000/36	SPAIR + SSGR + OFS	90/180	20	N/a	16.9/2.2	Dependent on current range covered	1	1	107	Dependent on current range covered
Single motor unit PGSE (single motor unit)	PGSE	160 × 160	1.5 × 1.5 × 8	2	16	1000/36	SPAIR + SSGR + OFS	90/180	20	N/a	16.9/2.2	540	1	1	107	09:00
PC for temporal muscle twitch (single motor unit)	PC Bipolar (4 acquisitions per slice velocity encoding flipped)	160 × 160	1.5 × 1.5 × 8	2	16	500/11.9	SPAIR + OFS	25	N/a	2	6.5/3.25	100	1	1	53	03:21

SPAIR = Spectral Adiabatic Inversion Recovery; SSGR = Slice Selective Gradient Reversal; OFS = Olefinic Fat Suppression.

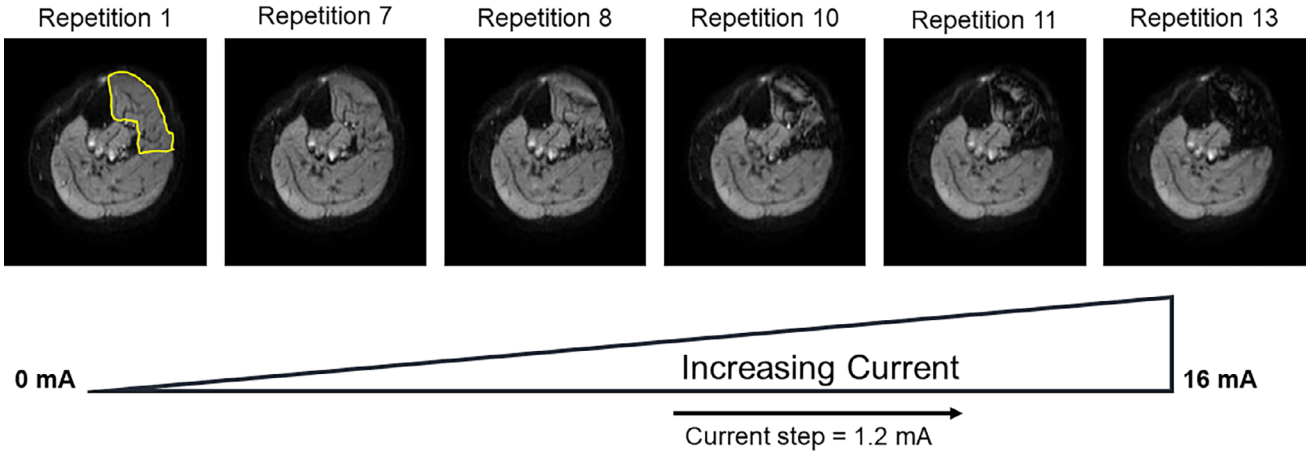


FIGURE 2: Example of a pulsed gradient spin echo set up scan. The peroneal nerve is stimulated leading to motor unit activity in the anterior compartment of the lower leg (delineated in yellow). From left to right, the stimulation current increases and with this the number of actively contracting motor units. Increased motor unit activity is reflected by increased blackness in the anterior compartment muscles (more areas with signal voids). In this example, the contrast is sufficient when the stimulation current reaches 16 mA, i.e. the whole anterior compartment is black and the muscle is visibly twitching. 16 mA would therefore be used as I_{muscle} in this participant.

with a single pulsed electrical stimulus at a magnitude of I_{muscle}, as this current leads to a full muscle twitch of the anterior muscle compartment. The stimulus onset was shifted relative to the motion sensitive window of the MRI sequence in steps of 5 msec (from 50 msec before the radiofrequency pulse of the MRI pulse sequence to 450 msec after the radiofrequency pulse; Fig. 3a). This scan was called the PC twitch profile scan. An example PC twitch profile scan is shown in Fig. 3b and Video S1. A region of interest (ROI) was drawn around the anterior compartment on the magnitude image (inset, Fig. 3b), this was performed by MB (MRI Physicist with >7 years' experience), after this the ROI outlines were then checked by AB (MR Physicist with >35 years' experience) and a consensus ROI was agreed upon. The ROI was then applied to the phase image and the average velocity in (cm/second) across the ROI for each repetition was measured and plotted against the time post stimulus. This process was also repeated for each individual muscle within the anterior compartment (tibialis anterior [TA], extensor digitorum longus [EDL], and peroneus longus [PL]).

Single MUs

In order to find the electrical stimulation current that activates only a single MU, a set of scans were acquired using a protocol of ramped electrical stimulation and a PGSE sequence. For the full MRI protocol, the reader is directed to the original paper.[17] For the single MU scan, a current step of 0.02 mA was used rather than the 0.01 mA from the original paper, this scan acquired 540 repetitions, the acquisition time was 9 minutes (Table 1).

The temporal shape of the twitch of the first active single MU was then estimated using the PC MRI sequence parameters are given in Table 1. An example single MU PC latency scan is shown in Video S2.

MRI Data Analysis

Contraction and Relaxation Times—Anterior Compartment and Individual Muscles

The contraction and relaxation times of the whole muscle twitch were calculated from the PC measured velocity profiles using custom-written analysis tools in Matlab 2021a (Mathworks, Natick, MA, USA). The data for each velocity profile were first baseline corrected.[13] Each curve was then displayed and the user was prompted to visually select the start of the increase in velocity ($Twitch_{start}$), the point at which the velocity signal crossed through zero on the x axis ($Twitch_{zerocross}$) and the end of the velocity signal ($Twitch_{end}$) (where the signal visually returned to baseline). This analysis was performed by MB (an MRI Physicist with >7 years' experience). These points are shown by the red markers in Figs. 3 and 4a–d.

The contraction and relaxation times were then calculated using Eqs. 1 and 2:

$$\text{Contraction time (msec)} = \text{Twitch}_{\text{zerocross}} - \text{Twitch}_{\text{start}}. \quad (1)$$

$$\text{Relaxation time (msec)} = \text{Twitch}_{\text{end}} - \text{Twitch}_{\text{zerocross}}. \quad (2)$$

Contraction and Relaxation Times—Single MUs

The twitch profile of the first active single MU was determined by first extracting a single MU ROI as described in Birkbeck et al.[17] This ROI was then applied to single MU PC twitch profile images to extract that single MU's velocity profile. Contraction and relaxation times of that single MU were then estimated using the method described above. Only one MU was studied per participant.

Statistical Analysis

A multivariable linear regression was used to test if participant age and sex significantly predicted contraction and relaxation times for the whole anterior compartment; the fitted regression model was: (contraction/relaxation time $= \beta_0 + \beta_1 \times$ [Age] $+ \beta_2 \times$ [Sex]). The relationships between participant age and muscle twitch characteristics from the individual muscles, and single MUs were also examined with a Pearson correlation.

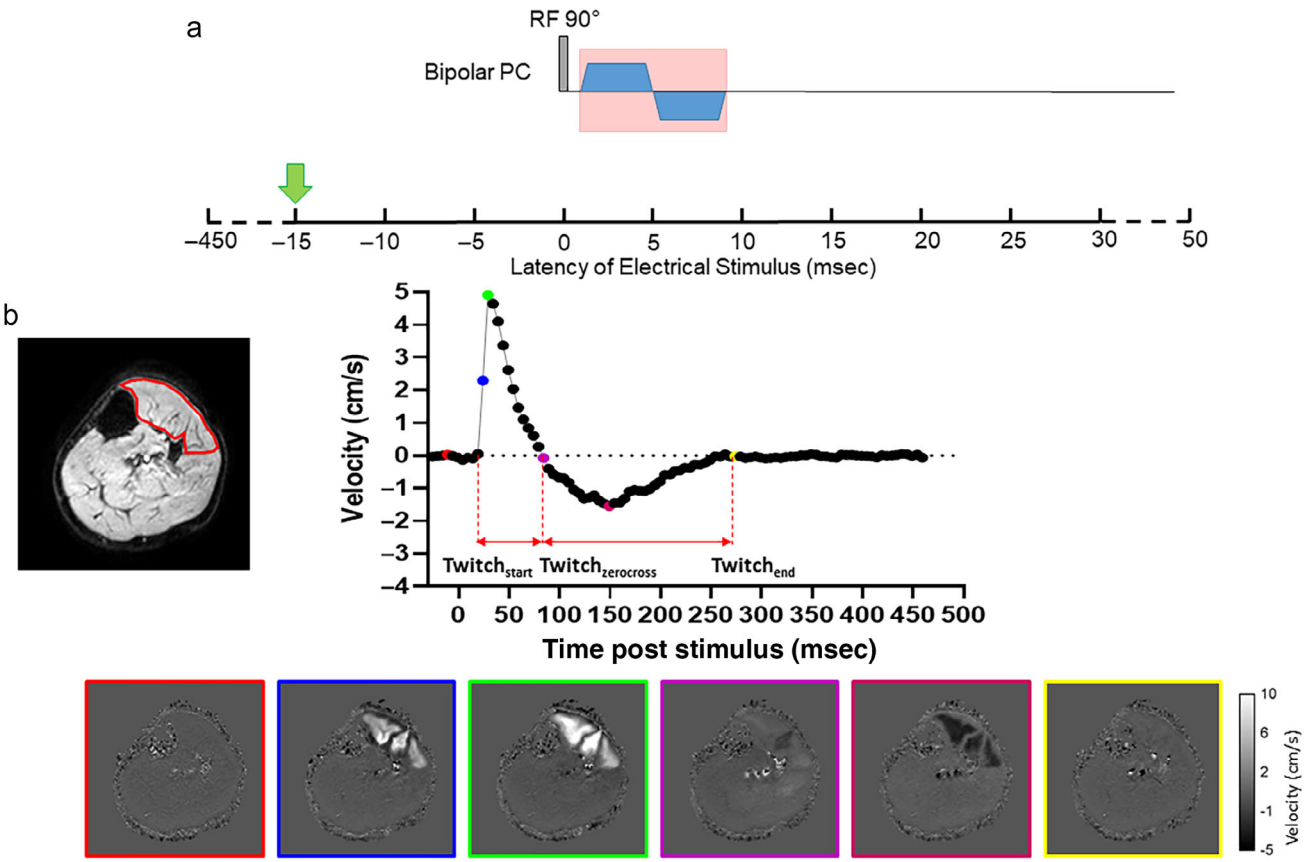


FIGURE 3: (a) Schematic overview of the phase contrast (PC) twitch profile scan showing how the timing of electrical stimulus (green arrow) was moved relative to the 90° RF pulse and motion sensitive window (the gradient in blue, delineated by the red rectangle) of the PC sequence. The stimulus current (I_{muscle}) was applied for each image with a different offset relative to the 90° RF pulse, from 50 msec before the RF pulse to 450 msec after the RF pulse. (b) Inset shows magnitude image from the PC scan with the ROI drawn around the anterior compartment muscles (red). The ROI was then applied to the phase image and the average velocity in the ROI was measured for each repetition. The extracted velocity curve is plotted against time post stimulus, with the colored points corresponding to the phase images depicted below with the same colored borders. For example, red represents a time-point before muscle contraction (no motion), green represents the time-point of maximum contraction velocity, pink represents the time-point of maximum relaxation velocity and yellow represents the time-point at the end of the twitch (no motion). The red arrows represent the points used to calculate the contraction and relaxation times using Eqs. 1 and 2.

Finally, relationships between participant age and measures of muscle strength: hand grip strength and MVC were assessed using Pearson correlation. All statistical tests were performed in GraphPad Prism for Windows, version 9.0.0 (GraphPad Software, San Diego, California, USA). Data are presented as mean ± standard deviation unless otherwise stated. A *P*-value <0.05 was set to be significant.

Results

Participant Characteristics

Sixty one participants (N = 32 male) were recruited into the study. Participants had a mean age of 55 ± 16 years (range: 26–82 years). Participant demographics are shown in Fig. 5. The tables in Fig. 5 show the participants split into three age groups representing young (25–44 years), middle (45–64 years), and old (65–85 years) participants. A total of 14 participants (N = 8 male) were excluded from the assessment of twitch characteristics from the whole anterior compartment and its constituent muscles. Participants were excluded because not all muscles in the anterior compartment were active on the PC twitch profile scan (N = 9), the contact of the stimulation electrodes was not adequate to elicit an observable muscle twitch (N = 2) or the twitch velocity exceeded the velocity encoding limit set in the MRI sequence (N = 3).

A total of 27 participants (N = 14 male) were excluded from the assessment of twitch characteristics of single MUs, because the resulting velocity profile from the PC twitch profile scan was too noisy or the single MU alternated during the scan making it difficult to estimate either contraction or relaxation time for the single unit (N = 15), the MU could not be detected in the PC sequence (N = 11), or equipment failure (N = 1). All of the 34 participants included in the assessment of single MU twitch characteristics were included in the group of 47 participants in which assessment of whole anterior compartment and individual muscle twitch characteristics was performed.

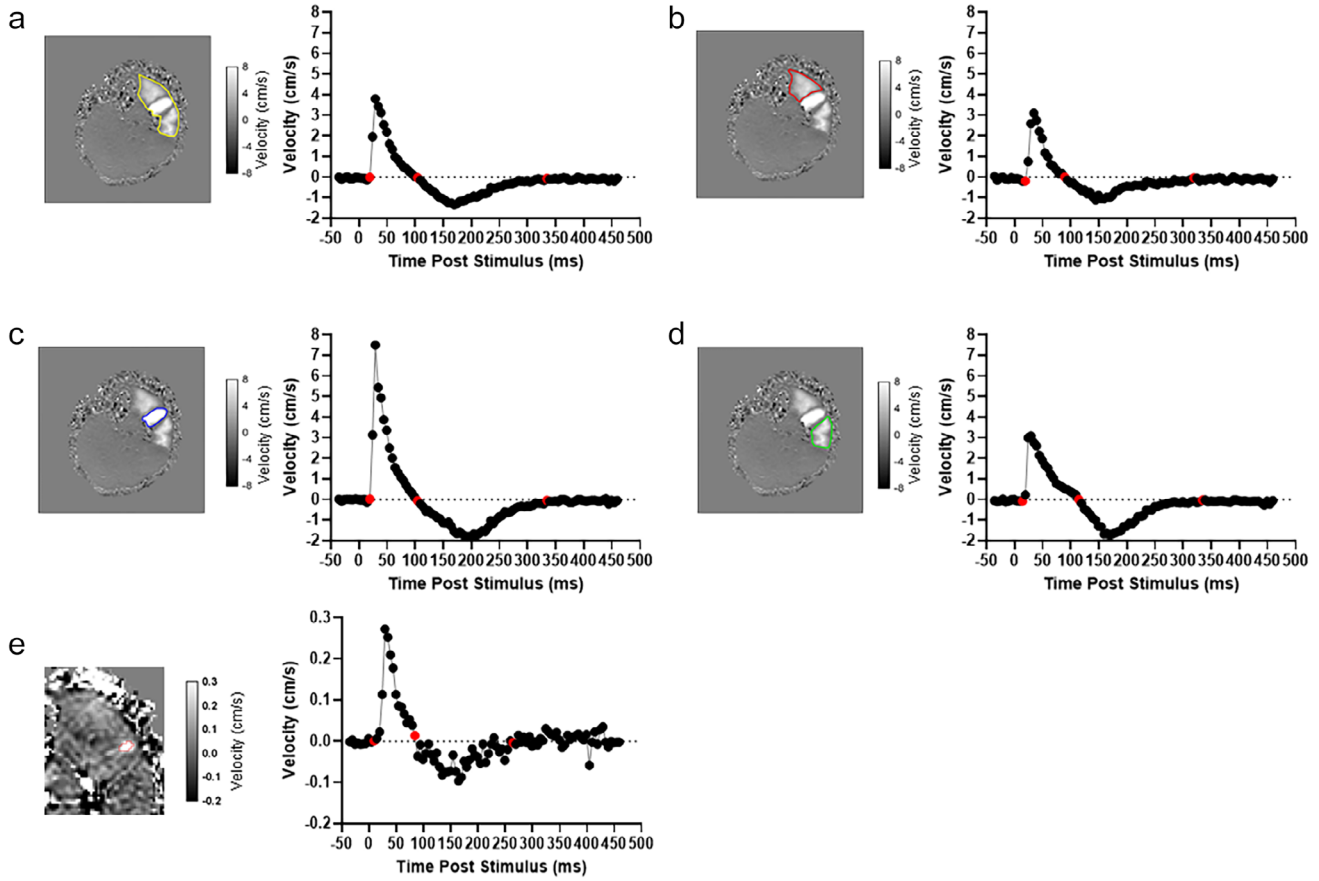


FIGURE 4: (a) Example velocity profile extracted from the whole anterior compartment (yellow ROI on inset phase image). The red points on each graph were used to calculate contraction and relaxation times. (b) Example velocity profile extracted from the tibialis anterior (red ROI on inset phase image). (c) Example velocity profile extracted from the extensor digitorum longus (blue ROI on inset phase image). (d) Example velocity profile extracted from the peroneus longus (green ROI on inset phase image). (e) Example velocity profile extracted from a single motor unit (red ROI on inset phase image).

Sarcopenia Screening

Across the 61 participants, the average grip strength in males (N = 32) was 41 ± 10 kg (range: 15–61 kg) and the average chair stand time in males was 13 ± 3 seconds (range: 7–20 seconds). The average grip strength in females (N = 29) was 28 ± 7 kg (range: 16–42 kg) and the average chair stand time in females was 13 ± 3 seconds (range: 7–18 seconds). None of the male or female participants fulfilled the criteria to indicate probable sarcopenia.

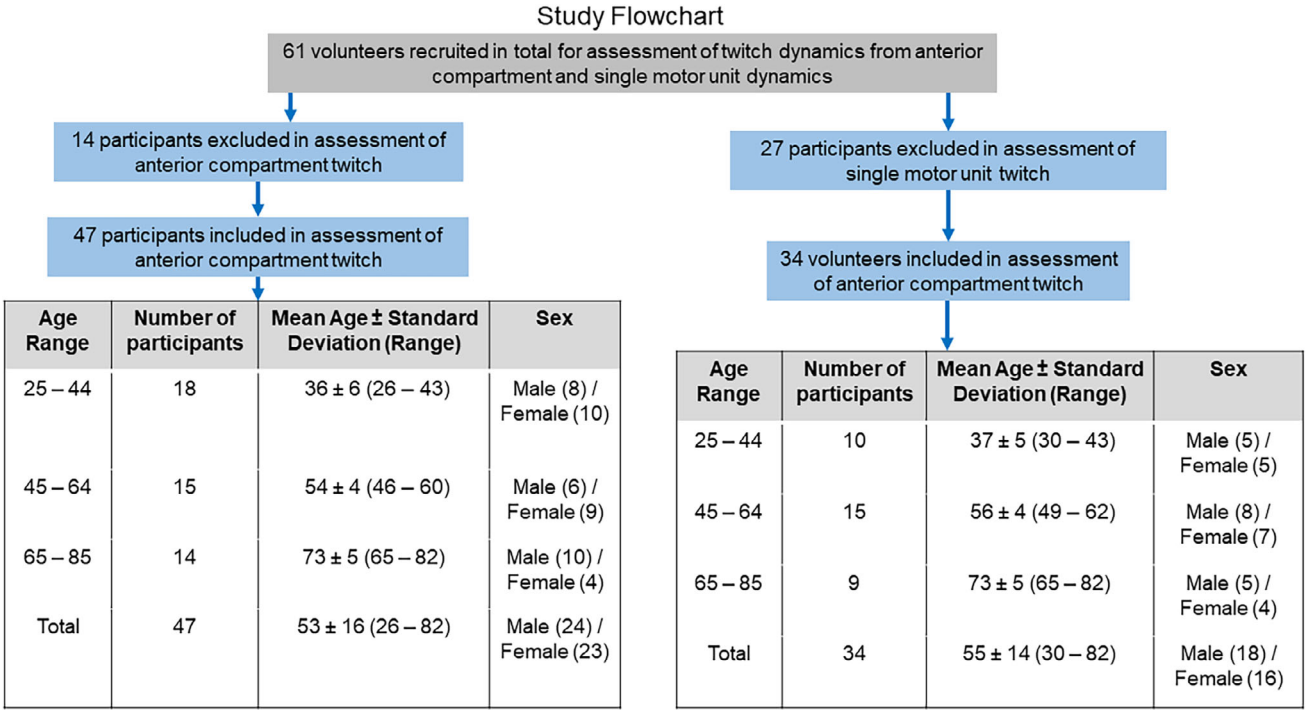
Relationship Between Age, Sex, and the Muscle Twitch Characteristics

Across the 47 participants the average contraction time of the anterior compartment was 83 ± 9 msec, with a range of 67 to 103 msec. The average relaxation time of the anterior compartment was 196 ± 36 msec, with a range of 132 to 287 msec. The average contraction time in females was 86 ± 8 msec compared to 80 ± 9 msec in males, contraction times were significantly longer in females than males. The average relaxation time in females was 204 ± 36 msec and 188 ± 34 msec in males, relaxation times were not different between the groups ($P = 0.151$).

Figure 6a shows the scatter plots for age vs. contraction and relaxation times for both males and females. For muscle contraction time the overall multivariable linear regression was statistically significant ($R^2 = 0.24$). Both age and sex significantly predicted contraction time. For muscle relaxation time the overall multivariable linear regression was statistically significant ($R^2 = 0.25$). Both age and sex significantly predicted relaxation time. Figure 6b shows the actual vs. predicted contraction and relaxation times from the model.

Individual Muscles

The contraction times for the TA, EDL, and PL were 76 ± 12 msec (range: 50–102 msec), 80 ± 11 msec (range: 58–103 msec) and 88 ± 12 msec (range: 62–114 msec), and did not correlate significantly with age ($P = 0.061$, $P = 0.186$, and $P = 0.168$, respectively) (Fig. 8). The relaxation times for the TA, EDL, and PL were 186 ± 33 msec (range: 132–261 msec), 178 ± 42 msec (range: 97–277 msec), and 195 ± 37 msec (range: 128–278 msec). The relaxation time of the TA and PL correlated significantly with age ($R^2 = 0.17$ and $R^2 = 0.24$, respectively) (Fig. 7a–f).



Age Range	Number of participants	Mean Age ± Standard Deviation (Range)	Sex
25 – 44	18	36 ± 6 (26 – 43)	Male (8) / Female (10)
45 – 64	15	54 ± 4 (46 – 60)	Male (6) / Female (9)
65 – 85	14	73 ± 5 (65 – 82)	Male (10) / Female (4)
Total	47	53 ± 16 (26 – 82)	Male (24) / Female (23)

Age Range	Number of participants	Mean Age ± Standard Deviation (Range)	Sex
25 – 44	10	37 ± 5 (30 – 43)	Male (5) / Female (5)
45 – 64	15	56 ± 4 (49 – 62)	Male (8) / Female (7)
65 – 85	9	73 ± 5 (65 – 82)	Male (5) / Female (4)
Total	34	55 ± 14 (30 – 82)	Male (18) / Female (16)

FIGURE 5: Study flowchart. A total of 61 volunteers were recruited to the study. Twitch dynamics of the lower leg anterior compartment and its individual muscles were measured in 47 volunteers and twitch dynamics of single motor units were measured in 34 volunteers. The tables show the participants split into three age groups: young (25–44 years), middle (45–64 years), and old (65–85 years) participants. The tables also show the number of participants in each age group and the mean age and sex.

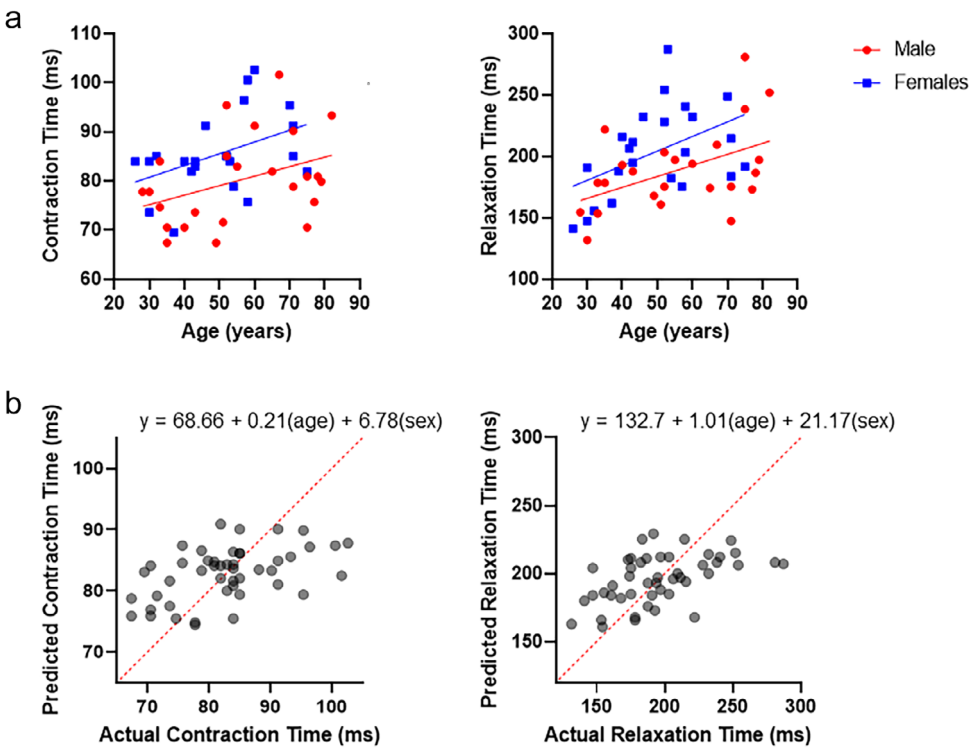


FIGURE 6: Effect of age and sex on contraction and relaxation times of the whole anterior compartment. (**a**) Left—Contraction time as a function of age for both male and female participants. Right—Relaxation time as a function of age for both male and female participants. (**b**) Left—Actual vs. predicted contraction time from multivariable linear regression. Right—Actual vs. predicted relaxation time from multivariable linear regression.

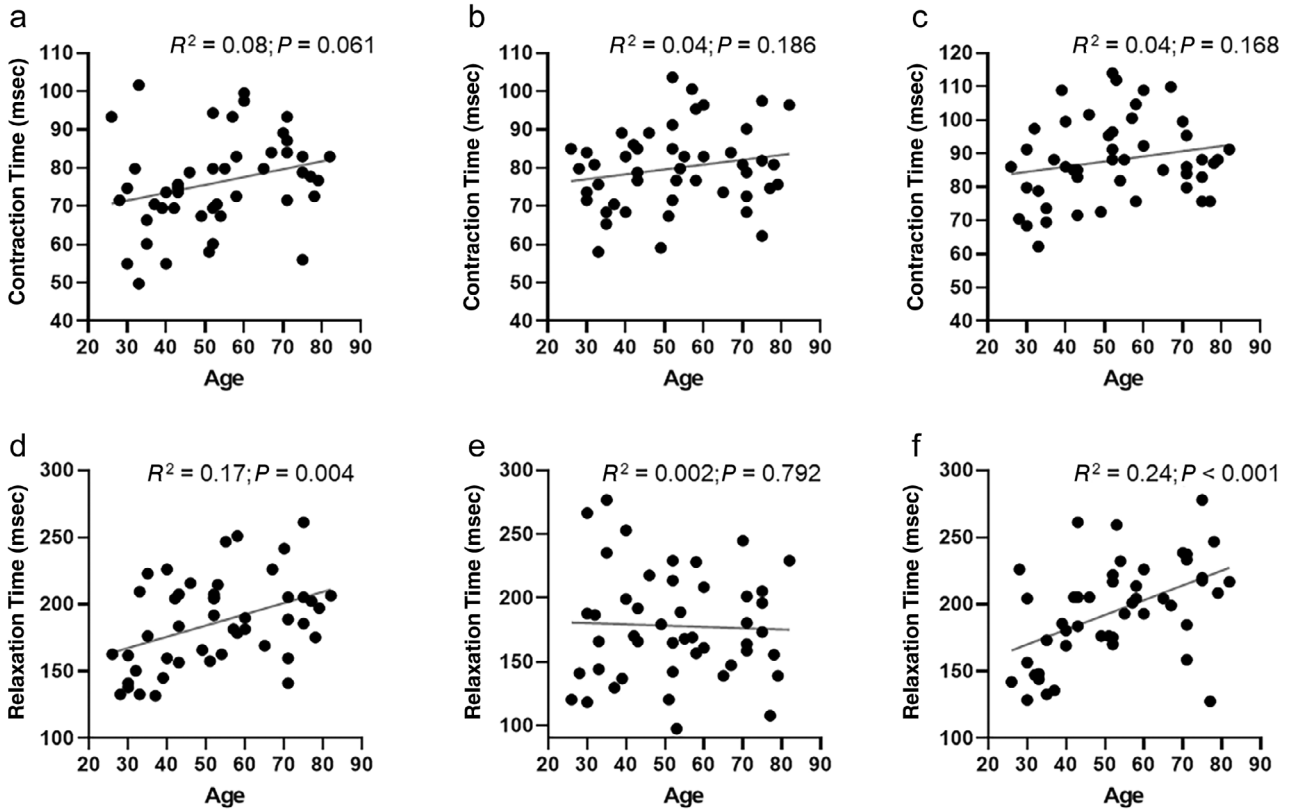


FIGURE 7: Effect of age on the twitch profile of individual muscles. (a) Muscle contraction time vs. age for the tibialis anterior. (b) Muscle contraction time vs. age for the extensor digitorum longus. (c) Muscle contraction time vs. age for the peroneus longus. (d) Muscle relaxation time vs. age for the tibialis anterior. (e) Muscle relaxation time vs. age for the extensor digitorum longus. (f) Muscle relaxation time vs. age for the peroneus longus.

Single MUs

Seventy four percent of observed units had contraction times of ≥80 msec and 62% of units had relaxation times of ≥200 msec (Fig. 8a). Due to the PL containing by far the most MUs, histograms show pooled data from all muscles and not individual muscles. The average contraction and relaxation times of a single MU measured from the velocity curve from the PC twitch profile scan were: 93 ± 31 msec (range: 31–158 msec) and 206 ± 52 msec (range: 66–273 msec), respectively. These contraction and relaxation times did not correlate significantly with age (contraction time: $R^2 = 0.02$, $P = 0.462$; relaxation time: $R^2 = 0.01$, $P = 0.534$) (Fig. 8b).

Muscle Twitch Properties and Muscle Functional Testing

Across the 47 participants included in the assessment of twitch characteristics in the anterior compartment, the mean hand grip strength was 35 ± 11 kg, range (15–61 kg) and the mean MVC was 23 ± 8 N, range (10–38 N). Contraction time was significantly negatively correlated with hand grip strength ($R^2 = 0.19$) and MVC ($R^2 = 0.11$) (Fig. 9a). Relaxation time was also significantly negatively correlated with hand grip strength ($R^2 = 0.09$) and MVC ($R^2 = 0.13$) (Fig. 9b). Contraction and relaxation times were positively related to chair stand times ($R^2 = 0.02$, $P = 0.345$ and $R^2 = 0.04$, $P = 0.180$, respectively).

Discussion

In this study, we measured the contraction and relaxation times of the whole lower leg anterior compartment, individual muscles, and single MUs using a PC MRI sequence. Relationships between participant age and whole lower leg anterior compartment, individual muscles, and single MU twitch profiles were investigated. When considering all three muscles of the anterior compartment acting as one group under an electrically induced stimulus, both the age and sex were found to be predictors of increased contraction and relaxation time, with females demonstrating longer contraction and relaxation times than males. The average contraction time (average 83 msec) and relaxation time (average 198 msec) measured in this study with PC MRI were in a similar range to those measured in previous studies stating approximately 100 msec contraction time and 200 msec relaxation time in leg extensor muscles using other techniques.[9,11,18–20]

Increases to contraction and relaxation times of muscle twitches with aging have been measured in both rodents and humans. Muscle time to peak contraction and relaxation

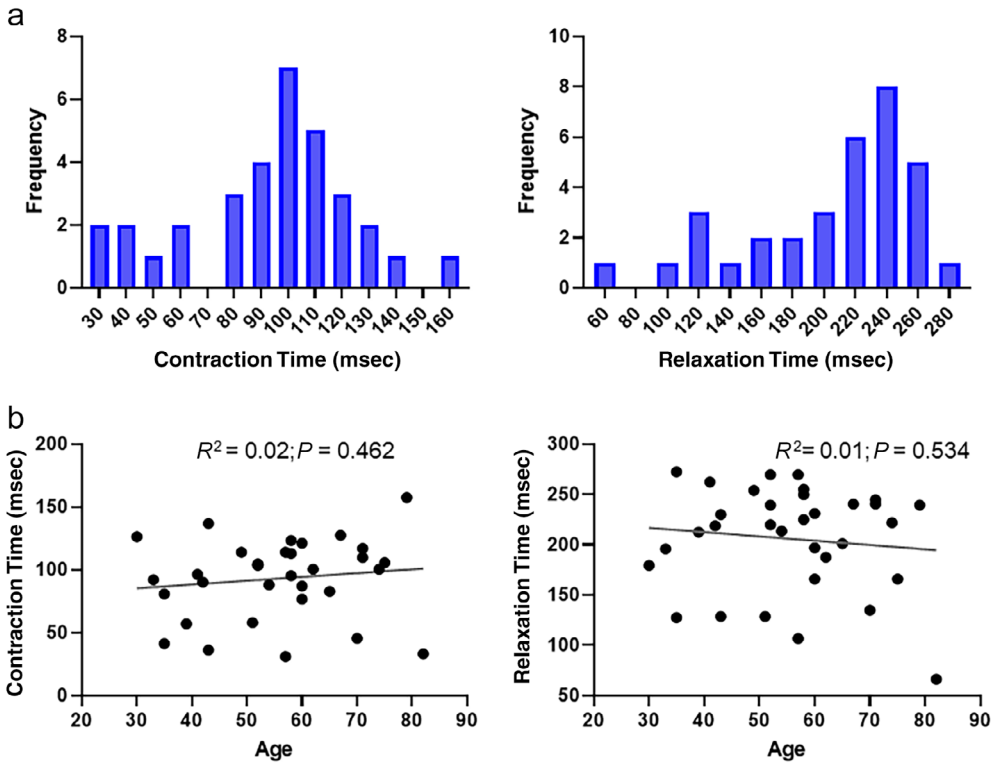


FIGURE 8: Contraction and relaxation times of single motor units. (a) Left—histogram of single motor unit contraction times. Right—histogram of single motor unit relaxation times. (b) Left—Single motor unit contraction times vs. age. Right—single motor unit relaxation times vs. age.

times of the extensor digitorum muscle were longer in aged mice than young mice.[21,22] In human studies changes to contraction and relaxation times at both the single fiber and whole muscle level have been found. Krivickas et al.[6] found that the maximum shortening velocity of skeletal muscle fibers from the vastus lateralis was significantly slower in type IIa fibers in older males and in type I fibers in older females. At the whole muscle level Pääuske et al.[7] demonstrated that twitch contraction times in plantarflexor muscles were significantly longer in older compared to younger females. These results support our findings that both contraction and relaxation times of the leg extensor muscles in the lower limb were significantly increased with aging. There are a number of suggestions as to why this occurs. First, the increase in contraction and relaxation times with age may be due to the bulk shift in muscle fiber type from fast twitch type II toward slower twitch type I fibers which is postulated to occur with aging.[23] Second, the chemical processes which facilitate muscle fiber contraction may be altered with age. For example it has been shown that aged muscle is less efficient at regulating the release and uptake of calcium ions Ca^{2+} which are a crucial determinant of the muscles contractile properties.[7,8,24] Third, degenerative changes to the connective tissues within skeletal muscles undergo stiffening with aging, which in turn leads to less elasticity in the muscle and slower contraction and relaxation times.[25] Finally, alterations to the electromechanical coupling (time delay between muscle activity and production of force at the tendon) of muscles may result in longer contraction and relaxation times in aged muscle.[26]

Results from this study suggest that females have significantly longer contraction and longer relaxation times (differences did not reach significance) than males. This has also been observed in rodents,[22] with female rodents having significantly longer twitch contraction times than males. This observation of longer contraction times in aged female rodents is thought to be due to falling levels of ovarian hormones in mice beyond 11 weeks old.[22] Whether this effect has a role in human studies remains unknown. In human studies, it has been suggested that estrogen effects Ca^{2+} kinetics.[6,27] With a reduction in estrogen post menopause, this may explain why older females demonstrated the longest contraction and relaxation times. Furthermore, Häkkinen et al.[20] demonstrated in leg extensor muscles that there was no significant change in relaxation time in voluntary isometric contraction between young and old human females. However, they also showed that the absolute maximal rate of relaxation was significantly smaller in older females aged >70 years. This was thought to reflect the reduction in cross sectional area of muscle with aging.

In order to assess if the observed changes in contraction and relaxation time accompany a change in the physical performance of muscle, we compared our measured contraction and relaxation times to hand grip strength and MVC of the lower limb muscles. Both of these clinically accepted

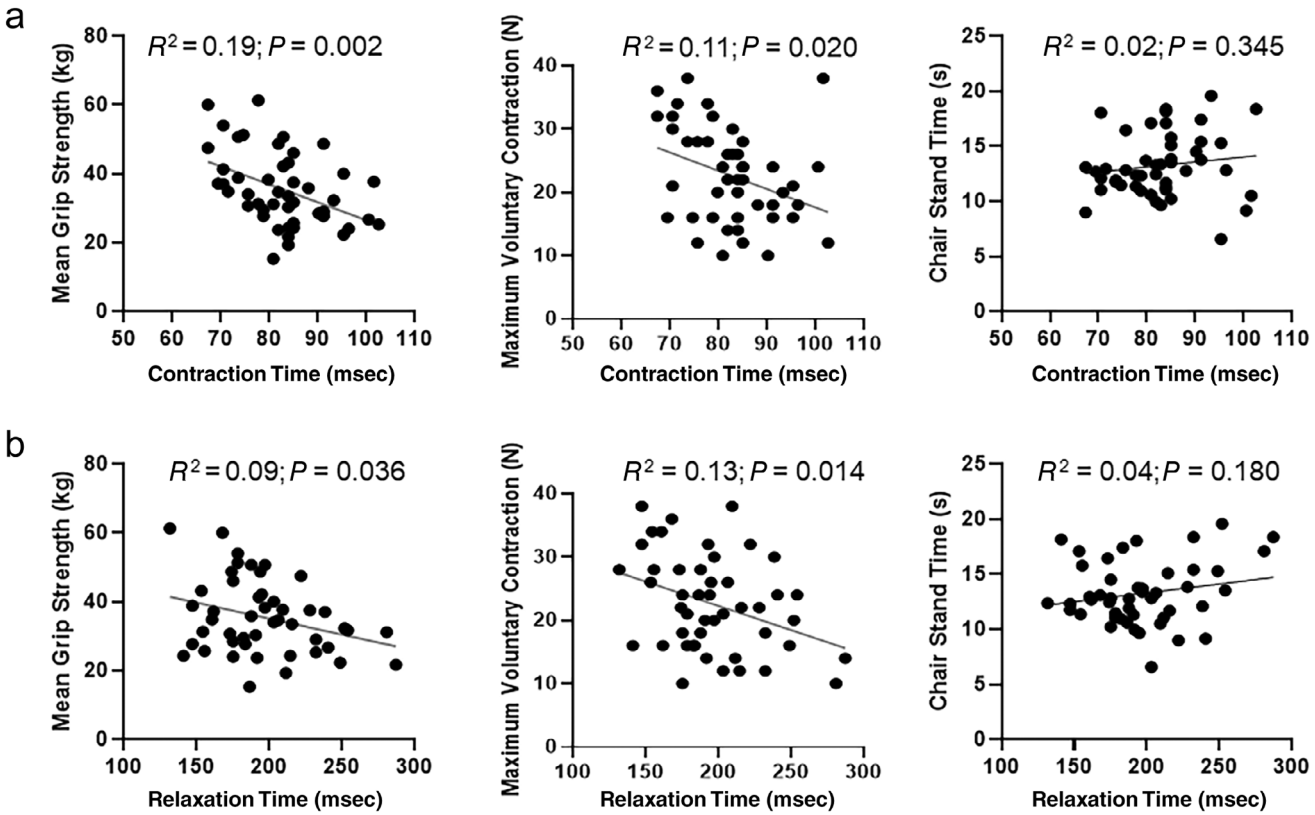


FIGURE 9: Correlations between contraction and relaxation times and muscle strength measurements. (a) Left—Mean grip strength from participant's dominant hand vs. contraction time. Middle—Mean maximum voluntary contraction of the ankle dorsiflexors vs. contraction time. Right—Chair stand time vs. contraction time. (b) Left—Mean grip strength from participant's dominant hand vs. relaxation time. Middle—Mean maximum voluntary contraction of the dorsiflexors vs. relaxation time. Right—Chair stand time vs. relaxation time.

measures of muscle strength demonstrated strong correlations with the contraction and relaxation time data measured using PC-MRI. It has been demonstrated in numerous studies that peak muscle strength is reduced with age.[28,29] Data from our study suggest that metrics derived from PC-MRI correlate well with clinically relevant measures of muscle strength. A future randomized controlled trial could be used to reduce bias and establish if this is a causal relationship or an indirect effect of both of these muscle strength measures correlating with age.

One of the advantages of using the PC MRI technique compared to current electromyography techniques is that it allows accurate study of both individual muscle twitch profiles and single MU twitch profiles in-vivo. When studying individual muscles within the anterior compartment, the contraction times of the three muscles did not correlate significantly with age, although there was a slight increase in the contraction times with aging. In comparison, the relaxation times of the TA and PL muscles were correlated with age, with older participants having longer relaxation times in these muscles. The relaxation time of the EDL muscle was not correlated with age. One potential reason for these differences may be that these three muscles have different fiber types. The TA and PL muscles contain a higher proportion of slow twitch (type I) muscle fibers, approximately 73% and 63%, respectively[30] than the EDL with approximately 47% type I fibers. However, why the muscles which have the larger proportion of type I fibers showed the greatest change in contraction and relaxation times is unclear. Furthermore, this may also be due to the fact that the EDL is a difficult muscle to delineate at the current spatial resolution of 1.5 × 1.5 mm in plane and therefore, introduces errors in the ROI, leading to errors in the measured relaxation rate. It remains to be seen how individual muscle twitch characteristics differ in patients with a diagnosis of sarcopenia. In sarcopenia there is postulated to be a failure to re-innervate the lost fast twitch (type II) fibers,[4] therefore one may expect that sarcopenic muscle would present with slower contraction and relaxation times than muscle from healthy participants. This will be an important area for future research.

In this study, the contraction and relaxation times of single MUs were also measured. Unlike the whole muscle data, these data demonstrated no change with age. When considering the whole muscle data, the ROI encompassed all three active muscles within the anterior compartment which therefore, by default, included a mixture of MU types. The observed slowing of contraction and relaxation times with age could therefore be explained by the bulk shift of fast twitch

(type II) to slow twitch (type I) fibers which occurs during the aging process.[31] By contrast when we observe a single MU, we are isolating a particular MU type. The MUs in this experiment were activated through surface electrical stimulation applied over the peroneal nerve. It has been shown that motor recruitment with electrical stimulation applied over the nerve produces a non-physiological and potentially random pattern of MU recruitment.[32] Therefore, in this small sample size, this may explain why no differences were observed in the single MU data. When comparing this to data from the whole anterior compartment where higher stimulus currents were used and more of the MU population was recruited, this may also explain why the lengthening of contraction and relaxation time with aging was seen in the whole muscle approach but not in the single MU approach.

While the experiments were relatively complex to perform, an advantage of this technique is its ability to noninvasively study twitch characteristics from single MUs. This technique could be studied against qualitative and quantitative MRI techniques (eg Dixon and STIR imaging) to further our understanding of the changes to the neuromuscular system which occur with aging and lead to an overall loss of muscle strength and mass. It would be of interest to apply this technique in a cohort of patients with sarcopenia to investigate how muscle kinetics differ between healthy aging muscle and pathological muscle. Furthermore, the PC-MUMRI technique can in theory be applied to any muscle group which can be stimulated to study twitch dynamics in a range of different muscle groups and MU types.

Limitations

This study had a small sample size. The complexity of the measurements led us to exclude ~50% of the data. Exclusions were performed for a number of reasons but the most common was that the despite saturating the signal on the PGSE sequence the level of stimulus did not produce enough activity in the PC scan to include the whole anterior compartment in the analysis. This could be addressed by optimizing the VENC for individual participant to ensure that the level of signal change is sufficient for analysis. Similarly with single MUs the VENC could be optimized to ensure that the region is more clearly visible on the scans. For this reason, we kept our analysis of the single MU purely exploratory and assessed only simple Pearson correlations. The measured average contraction time agreed with previous studies performed using the same technique.[13] In addition, interestingly, the range of measurements suggested that the technique may be sensitive to discriminate between MUs with fast contraction and slow contraction times. Therefore, the PC technique provides a potential way to discriminate MU type, although this would need confirmation with animal studies of ex-vivo MUs.

A further limitation is that the muscle was not fully activated to compound muscle action potential as, with the current scan settings, this would have caused the phase data to alias and become corrupted. Furthermore, a number of participants had to be removed from the study due to either aliasing in the data or, in particular in the case of the single MU study, because the data were too noisy. Finally, as it has been shown that the TA is relatively spared in the aging process,[33] it would be of interest in future studies to apply this method in muscles where a greater reduction in type II fibers would be expected with age such as the Vastus Lateralis.[34] Further refinement of the MRI protocol is underway and we aim to be able to stimulate the muscle at supramaximal stimulus currents which would allow for a more standardized approach between participants and extend the technique to other muscle groups.

Finally, when modeling the effects of age and sex on contraction and relaxation times of the whole anterior compartment, the linear multivariable model predicts the contraction and relaxation times within a given range, however, for contraction times <75 msec or >90 msec this prediction begins to fall down. In order to improve the prediction of the model, it will be necessary to acquire more data using the PC-MUMRI technique.

Conclusions

In this study, a novel MRI technique was used to investigate the relation between contraction and relaxation times of healthy muscle change with age. Experiments to measure the contraction and relaxation times in the lower leg anterior compartment, individual muscles and single MUs were performed in a large cohort of healthy aging volunteers. Future studies should involve the collection of PC-MUMRI data in participants with a diagnosis of Sarcopenia to compare to the data from this study.

Acknowledgments

This work was funded by the NIHR Newcastle Biomedical Research Centre. The NIHR Newcastle Biomedical Research Centre is a partnership between Newcastle Hospitals NHS Foundation Trust and Newcastle University, funded by the National Institute for Health and Care Research (NIHR). This paper presents independent research funded and supported by the NIHR Newcastle Biomedical Research Centre. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

Conflict of Interest

The authors have no conflicts of interest to disclose.

References

1. Purves D, Augustine GJ, Fitzpatrick D. *Neuroscience: The motor unit.* 2nd ed. Sunderland (MA): Sinauer Associates; 2001.

2. Kadhiresan V, Hasset C, Faulkner J. Properties of single motor units in medial gastrocnemius muscles of adult and old rats. J Physiol 1996; 493(2):543-552.
3. Jones EJ, Chiou S, Atherton PJ, Phillips BE, Piasecki M. Ageing and exercise-induced motor unit remodelling. J Physiol 2022;600(8):1839-1849.
4. Piasecki M, Ireland A, Piasecki J, et al. Failure to expand the motor unit size to compensate for declining motor unit numbers distinguishes sarcopenic from non-sarcopenic older men. J Physiol 2018;569(9): 1627-1637.
5. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: Revised European consensus on definition and diagnosis. Age Ageing 2019;48(1):16-31.
6. Krivickas L, Dongwon S, Wilkins J, Hughes VA, Roubenoff R, Frontera W. Differences in maximum shortening velocity of skeletal muscle fibers. 2001. pp. 447–455.
7. Pääsuke M, Ereline J, Gapeyeva H, Sirkel S, Sander P. Age-related differences in twitch contractile properties of plantarflexor muscles in women. Acta Physiol Scand 2000;170:51-57.
8. Verschueren A, Palminha C, Delmont E, Attarian S. Changes in neuromuscular function in elders: Novel techniques for assessment of motor unit loss and motor unit remodeling with aging. Rev Neurol (Paris) 2022;178(8):780-787.
9. Leitch M, Macefield VG. Comparison of contractile responses of single human motor units in the toe extensors during unloaded and loaded isotonic and isometric conditions. J Neurophysiol 2015;114(2):1083-1089.
10. Cogliati M, Cudicio A, Martinez-Valdes E, et al. Half marathon induces changes in central control and peripheral properties of individual motor units in master athletes. J Electromyogr Kinesiol 2020;55(May):102472.
11. Sanchez GN, Sinha S, Liske H, et al. In vivo imaging of human sarcomere twitch dynamics in individual motor units. Neuron 2015;88(6): 1109-1120.
12. Wymer DT, Patel KP, Burke WF, Bhatia VK. Phase-contrast MRI: Physics, techniques, and clinical applications. Radiographics 2020;40(1): 122-140.
13. Heskamp L, Birkbeck MG, Whittaker RG, Schofield IS, Blamire AM. The muscle twitch profile assessed with motor unit magnetic resonance imaging. NMR Biomed 2021;34(3):e4466.
14. Dodds RM, Hurst C, Hillman SJ, et al. Advancing our understanding of skeletal muscle across the lifecourse: Protocol for the MASS_Lifecourse study and characteristics of the first 80 participants. Exp Gerontol 2022;166:111884.
15. Roberts HC, Denison HJ, Martin HJ, et al. A review of the measurement of grip strength in clinical and epidemiological studies: Towards a standardised approach. Age Ageing 2011;40(4):423-429.
16. Steidle G, Schick F. Addressing spontaneous signal voids in repetitive single-shot DWI of musculature: Spatial and temporal patterns in the calves of healthy volunteers and consideration of unintended muscle activities as underlying mechanism. NMR Biomed 2015;28:801-810.
17. Birkbeck MG, Heskamp L, Schofield IS, Blamire AM, Whittaker RG. Non-invasive imaging of single human motor units. Clin Neurophysiol 2020;131(6):1399-1406.
18. Garnett RA, O'Donovan MJ, Stephens JA, Taylor A. Motor unit organization of human medial gastrocnemius. J Physiol 1979;287:33-43.
19. Andreassen S, Arendt-Nielsen L. Muscle fibre conduction velocity in motor units of the human anterior tibial muscle: A new size principle parameter. J Physiol 1987;391:561-571.
20. Häkkinen K, Häkkinen A. Muscle cross-sectional area, force production and relaxation characteristics in women at different ages. Eur J Appl Physiol Occup Physiol 1991;62(6):410-414.
21. Fitts RH, Troup JP, Witzmann FA, Holloszy JO. The effect of ageing and exercise on skeletal muscle function. Mech Ageing Dev 1984; 27(2):161-172.
22. Chan S, Head SI. Age- and gender-related changes in contractile properties of non-atrophied EDL muscle. PLoS One 2010;5(8):20-22.
23. St-Jean-Pelletier F, Pion CH, Leduc-Gaudet J-P, et al. The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men. J Cachexia Sarcopenia Muscle 2017;8(2):213-228.
24. Lamboley CR, Wyckelsma VL, Dutka TL, McKenna MJ, Murphy RM, Lamb GD. Contractile properties and sarcoplasmic reticulum calcium content in type I and type II skeletal muscle fibres in active aged humans. J Physiol 2015;593(11):2499-2514.
25. Agyapong-Badu S, Warner M, Samuel D, Stokes M. Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device. Arch Gerontol Geriatr 2016;62:59-67.
26. Libardi CA, Souza TMF, Conceição MS, et al. Electromechanical delay of the knee extensor muscles: Comparison among young, middle-age and older individuals. Clin Physiol Funct Imaging 2015;35(4):245-249.
27. Sarwar R, Niclos BB, Rutherford OM. Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle. J Physiol 1996;493(1):267-272.
28. Power GA, Dalton BH, Rice CL. Human neuromuscular structure and function in old age: A brief review. J Sport Heal Sci 2013;2(4):215-226.
29. Dodds RM, Syddall HE, Cooper R, et al. Grip strength across the life course: Normative data from twelve British studies. PLoS One 2014; 9(12):1-15.
30. Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. J Neurol Sci 1973;18(1):111-129.
31. Larsson L. Chapter 8 effects of aging on motor unit structure and function. Handb Clin Neurophysiol 2003;2:119-144.
32. Bergquist AJ, Clair JM, Collins DF. Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: Triceps surae. J Appl Physiol 2011;110(3):627-637.
33. Höök P, Sriramoju V, Larsson L. Effects of aging on Actin sliding speed on myosin from single skeletal muscle cells of mice, rats, and humans. Am J Physiol Cell Physiol 2001;280(4):782-788.
34. Lexell J, Henriksson-Larsén K, Sjöström M. Distribution of different fibre types in human skeletal muscles. 2. A study of cross-sections of whole m. vastus lateralis. Acta Physiol Scand 1983;117(1):115-122.