Bi-component T1\(\rho\) and T2 Relaxation Mapping of Skeletal Muscle In-Vivo

Azadeh Sharafi, Gregory Chang & Ravinder R. Regatte

The goal of this paper was to evaluate the possibility of bi-component T1\(\rho\) and T2 relaxation mapping of human skeletal muscle at 3T in clinically feasible scan times. T1\(\rho\)- and T2-weighted images of calf muscle were acquired using a modified 3D-SPGR sequence on a standard 3T clinical MRI scanner. The mono- and biexponential models were fitted pixel-wise to the series of T1\(\rho\) and T2 weighted images. The biexponential decay of T1\(\rho\) and T2 relaxations was detected in ~30% and ~40% of the pixels across all volunteers, respectively. Monoexponential and bi-exponential short and long T1\(\rho\) relaxation times were estimated to be 26.9 ms, 4.6 ms (fraction 22%) and 33.2 ms (fraction: 78%), respectively. Similarly, the mono- and bi-exponential short and long T2 relaxation times were 24.7 ms, 4.2 ms (fraction 15%) and 30.4 ms (fraction 85%) respectively. The experiments had good repeatability with RMSCV < 15% and ICC > 60%. This approach could potentially be used in exercise intervention studies or in studies of inflammatory myopathies or muscle fibrosis, permitting greater sensitivity and specificity via measurement of different water compartments and their fractions.

Skeletal muscle is a very heterogeneous tissue, which composed of different types of muscle fiber. In most cases, the muscular disease affects the properties of the muscle fibers and as a result, changes their relaxation times. For example, in muscle fibrosis, the damaged striated skeletal muscle is replaced mainly by excessive collagen. As the disease progresses, the water content of the muscle will increase, leading to a lengthening of the relaxation time. Hence, monitoring relaxation times such as T2 and T1\(\rho\) as noninvasive biomarkers may provide valuable information on the disease progression. Monoexponential measurement of T1\(\rho\) and T2 was investigated for disease monitoring and the elevated T1\(\rho\) and T2 due to a disease or an injury have been shown in several studies. Recently, Iijima et al. showed the increase of T2 relaxation time in rotator cuff muscles with the extent of the tear. Moreover, Maillard et al. proposed using T2 relaxation as a quantitative measure of muscle inflammation. In another study, Hatakenaka et al. showed the effect aging on T2 relaxation time. However, as shown by Hazlewood et al. different fractions of non-(or slowly) exchanging water exists in the muscle tissues. Hence; a multieexponential model may better present the relaxation time components in the muscle. Saab et al. reported the multieexponential behavior of T2 relaxation in skeletal muscle. More recently, Araujo et al. proposed a method to measure the short T2 component in skeletal muscle (SKM) in the presence of fat using a UTE sequence. Moreover, the biexponential behavior of T1\(\rho\) relaxation has been observed in rat muscle.

To the best of our knowledge, the biexponential measurement of T1\(\rho\) and T2 has yet to be reported in vivo. The purpose of this work is to evaluate the in-vivo feasibility of biexponential analysis of T1\(\rho\) and T2 relaxation times of human calf muscle using 3T MRI in clinically feasible scan times.

Results
Monte Carlo Simulation. As shown in Fig. 1, higher SNR leads to more accurate estimation (Fig. 1a). The SNR of the in-vivo T1\(\rho\) and T2 experiments was 65 ± 11 and 76 ± 13, respectively; hence about [12–16%] (T1\(\rho\)) and [9–14%] (T2) estimation error was expected for in-vivo estimation of short relaxation time, and about [5–10%] error is expected in estimating the long relaxation time as well as the short and long fractions. The number of time points (TSL or TE) also affects the estimation. As shown in Fig. 1b, acquiring data in more time points increases the fitting accuracy and as a result a better relaxation estimation (Fig. 1b). However, the acquisition time...
increases linearly with the number of points, so a trade-off must be made between image acquisition time and measurement accuracy. As shown in Fig. 1b, the improvement from 10 to 15-time points is negligible (less than 2%) considering the 50% increase in the total scan time. Hence, in this study, 10 TSL/TE points were selected for the in-vivo experiment. As shown Fig. 1c and d, the estimation errors are higher for shorter long and longer short components. The effect of component fractions on the estimation is shown in Fig. 1e and f. The component with higher fraction is estimated more accurately than the one with lower fraction.

In vivo experiment. Figure 2 shows a representative slice of a scan with TSL = 2 ms in axial, sagittal, and coronal planes and the regions of interest (ROIs) in which the relaxation components were estimated. A representative example of T1ρ and T2 maps are shown in Fig. 3a1–d1 and Fig. 3a2–d2 respectively. The summary of descriptive statistics calculated across eight participants in each ROI is summarized in Table 1. The estimated monoexponential and biexponential short and long T1ρ over different regions were varied between [23.7 ms, 31.4 ms], [3.5 ms–13.5 ms] and [28.1 ms–41.3 ms], respectively; while the T2 mono, short and long components ranged from [18.5 ms–27.8 ms], [4.2 ms–8.7 ms] and [28.4–55.2 ms], respectively. The short component has lower fraction than the long relaxing component. In this study, we observed that a biexponential fit is a better describes the T1ρ and T2 relaxation decay than a monoexponential model. As shown in Fig. 4a, since the monoexponential fit appears as a straight line in logarithmic scale, the deviation of the data points from the line indicates the existence of more than one exponential term in the model. Moreover, the biexponential fit has smaller residuals than the monoexponential fit which confirm that it can better represent the relaxation decay.

Statistical Analysis. Figure 5 shows the comparison between T1ρ and T2 relaxation time in different muscle ROIs. The Wilcoxon rank sum test results showed that the global mono and long T1ρ relaxation components were significantly higher than T2 relaxation. The gender difference analysis results for T1ρ and T2 relaxation components revealed that the short T2 relaxation component was significantly greater in male participants than the female participants. In addition, the Kruskal-Wallis test was applied to investigate the difference between different ROIs. The results showed that there is a statistically significant difference in monoexponential T1ρ (P < 0.001).
and T2 (P = 0.0038) in different ROIs. No significant difference was observed in biexponential components. The pairwise comparison between ROIs is shown in Table 2.

Figure 6 shows the ICC and RMSCV across three participants. The ICC > 60% and RMSCV < 15% on all the regions show the good reliability and repeatability of this study.

Discussion
In this paper, we presented a 3T MRI technique for in-vivo, bi-component T1ρ and T2 analysis of calf muscle. Five ROIs were defined in the muscle and T1ρ and T2 relaxation times were measured in each ROI. The calf muscle chemical composition consists of intra- (~25%) and extracellular water (~75%), contractile proteins (~20%:......
myosin, actin, tropomyosin/troponin, myoglobin) and other components (~5%: salts, phosphates, ions, glycogen, and macronutrients). The short components are thought to be related to the tightly bound macromolecular (collagen, contractile proteins, and other components, etc.) and intracellular water compartments; while the long relaxation component corresponds mainly to the loosely bound water (extracellular/vascular). The results showed that biexponential fitting might better present and distinguish the different relaxation times in the muscle due to different water compartments. The estimated monoexponential relaxations were comparable to the other studies. The monoexponential estimated \( T_1 \) was higher than \( T_2 \). To the best of our knowledge this comparison has not been reported for the calf muscle, however, our results trend are in agreement with other tissues such as articular cartilages in which \( T_1 > T_2 \).

The existence of three relaxation components was shown in previous studies using Carr-Purcell-Meiboom-Gill (CPMG) sequence. Saab et al. reported the in vivo multi-component \( T_2 \) relaxations in flexor digitorum profundus muscle while Cole et al. measured the \( T_2 \) relaxation in rat muscle. These three components have been related to the hydration shell of macromolecules, intracellular water, and extracellular water, respectively. In a study performed by Araujo et al. the existence of the biexponential relaxation behavior (e.g., an intermediate

| ROI | Relaxation Type | \( T_{mono} \) (ms) | \( T_{short} \) (ms) | \( F_{short} \) (%) | \( T_{long} \) (ms) | \( F_{long} \) (%) | Ratio (%) |
|-----|----------------|---------------------|---------------------|-------------------|-----------------|-----------------|-----------|
| GM  | \( T_1p \)     | 28 ± 1.5            | 6.1 ± 0.6           | 21 ± 1.5          | 36 ± 2.3        | 79 ± 1.5        | 30 ± 0.1   |
|     | \( T_2 \)      | 25 ± 0.9            | 6.3 ± 0.7           | 18 ± 4.5          | 34 ± 2.6        | 82 ± 4.5        | 18 ± 0.0   |
| GL  | \( T_1p \)     | 28 ± 2.2            | 7.8 ± 2.9           | 25 ± 15           | 42 ± 11         | 75 ± 15         | 22 ± 0.1   |
|     | \( T_2 \)      | 26 ± 1.1            | 5.9 ± 0.8           | 15 ± 3.7          | 33 ± 3.5        | 85 ± 3.7        | 17 ± 0.1   |
| SOL | \( T_1p \)     | 29 ± 1.0            | 6.4 ± 0.6           | 21 ± 2.5          | 37 ± 2.3        | 79 ± 2.5        | 30 ± 0.1   |
|     | \( T_2 \)      | 26 ± 0.6            | 5.6 ± 0.3           | 16 ± 2.4          | 33 ± 1.1        | 84 ± 2.4        | 16 ± 0.0   |
| PER | \( T_1p \)     | 28 ± 2.6            | 6.2 ± 1.3           | 26 ± 10           | 37 ± 5.0        | 74 ± 10         | 32 ± 0.1   |
|     | \( T_2 \)      | 25 ± 2.2            | 5.9 ± 1             | 22 ± 15           | 35 ± 8.2        | 78 ± 15         | 20 ± 0.1   |
| TA  | \( T_1p \)     | 25 ± 1.0            | 6.2 ± 0.4           | 31 ± 5.8          | 37 ± 1.3        | 69 ± 5.8        | 28 ± 0.1   |
|     | \( T_2 \)      | 23 ± 2.2            | 6.8 ± 1.0           | 30 ± 10           | 39 ± 5.3        | 70 ± 10         | 14 ± 0.1   |
| Global | \( T_1p \)  | 27 ± 1.2            | 6.3 ± 0.3           | 25 ± 2.7          | 37 ± 1.5        | 75 ± 2.7        | 29 ± 0.0   |
|     | \( T_2 \)      | 25 ± 1.0            | 6.1 ± 0.5           | 20 ± 3.6          | 35 ± 2          | 80 ± 3.6        | 17 ± 0.0   |

Table 1. Summary of \( T_1p \) and \( T_2 \) relaxation times estimation in 5 different regions of interest.

Figure 4. Mono- and bi-exponential fit comparison. (a1,b1) \( T_1p \) and (a2,b2) \( T_2 \) decay and the fit residuals in representative voxels.
and a long components) has been confirmed for T2 relaxation time using a localized 2D-ISIS-CPMG sequence. However, no short component has been detected due to long TE’s used in the sequence24. In contrast, our method can measure a short component and provide 3D volumetric maps. Recently, Araujo et al.2 proposed a UTE sequence to measure the short T2 components. Only the short component was measured due to the short TEs used in this study. The total acquisition time to acquire one scan with 7 TE values was 7 min, 49 s. They measured T2 in only one thick (6 mm) slice while we acquired 3D volumetric scans. However, our method cannot detect very short T2 due to using TE of 3.78 ms in the readout.

Due to the SAR limitation in the T1ρ experiment, the longest TE/TSL in our study was 55 ms. Hence; our long component is close to the third component, and our short component is close to the first component calculated in Saab study. Moreover, the smaller slice thickness (2 ms) in our study in comparison with Saab study (10 mm) leads to lower partial volume effect (PVE).

To the best of our knowledge, biexponential T1ρ measurement of muscle has only been done on animals. For example, a biexponential analysis of T1ρ in rat muscles was reported by Yuan et al.15. The mono, short and long T1ρ were measured as (~30–33 ms), (~9–11 ms) and (~37–41 ms), respectively. The short and long fractions were (~12–20%) and (~80–88%). 25 temporal points from TSL = 1 ms to 60 ms were acquired in this study at the cost of increasing the total acquisition time to ~30 minutes15. Our T1ρ estimations using 10 TSLs acquired in 15 minutes scans are in good agreement with this study. The difference is probably due to the higher temporal resolution in Yuan study.

The selection of TR can affect the T1ρ and T2 estimation due to the T1 relaxation. We evaluated this effect using Bloch simulation. Our simulation showed that there is ~5% difference between the T1ρ or T2 estimation error with TR = 1500 ms and TR = 5000 ms. Considering the longer acquisition time of TR = 5000 ms, we considered this error as negligible.

The fatty infiltration of muscular occurring in some cases such as atrophy and muscular dystrophy does not affect the relaxation time since the fat signal was suppressed in the scans by exciting only the water with a binomial RF excitation pulse25,26.

Our study has some limitations. The biexponential condition of 4T_short < T_long can produce some bias. We chose this condition based on the suggestion in Juras study27.

Field inhomogeneities also affected the estimation since the spin-locking in T1ρ imaging is very sensitive to B₀ and B₁ inhomogeneities. To compensate this effect, as described in our previous studies28, we used spin-lock phase
alteration and a refocusing pulse for $B_1$ and $B_0$ compensation, respectively. In addition, the manual shimming was performed to further correct the field inhomogeneities. Our results showed a homogenous $B_0$ ($\Delta B_0 < \pm 5$ Hz) and $B_1$ changes less than $\pm 50$ Hz across ROIs. However, the compensation techniques used in this study may not be successful for scanning large volumes such as gluteus muscle or covering inhomogeneous regions such as arms and dorsal muscles. Moreover, the binomial RF excitation pulse is not robust in the presence of large inhomogeneities, and hence, the fatty infiltration may affect the estimation. Further studies in the large volumes muscles and, in the presence of fatty infiltration are warranted to evaluate the performance of our method.

The magic angle effect related to dipolar interactions of fiber orientation with respect to $B_0$ may affect the $T_{1\rho}$ and $T_2$ values. The spins decay monoexponentially when the tissue’s orientation to $B_0$ is about 55°29, though $T_{1\rho}$ is relatively less sensitive than $T_2$ to this effect29.

Finally, we expect an elevation in relaxation components due to a muscular disease such as muscle fibrosis. However, only a small number of asymptomatic participants were scanned in this study and further validation in patients with fibrosis is warranted.

In conclusion, in this study, we showed the feasibility of in vivo measurement of bi-exponential $T_{1\rho}$ and $T_2$ relaxation of human calf muscle in clinically feasible scan times. Our method could potentially be used in intervention exercise studies or in studies of inflammatory myopathies or muscle fibrosis, permitting greater sensitivity and specificity via measurement of different water compartments and their fractions.

Figure 6. Repeatability study (a) ICC (b) RMSCV in different muscle ROIs.
Methods

Monte Carlo Simulation. The signal decay during the spin-lock duration (TSL) or echo time (TE) can be defined as the summation of \( M \) exponential terms with different fractions \( (a_i) \) and relaxation time constants \( (T_i) \):

\[
S(t) = S_0 \sum_{i=1}^{M} a_i e^{-\frac{t}{T_i}} + N
\]  

(1)

where \( S(t) \) is the MRI signal intensity at time \( t \) (TSL in T1\( \rho \) and TE in T2 imaging), \( S_0 \) is initial value, \( a_i \) is the fraction of \( i \)th exponential term with the assumption of \( \sum_{i=1}^{M} a_i = 1 \), and \( N \) is the additive noise. Assuming \( S_0 = 1 \), to express biexponential decay \( (M = 2) \), the equation can be written as:

\[
S(t) = a_1 e^{-\frac{t}{T_1}} + a_2 e^{-\frac{t}{T_2}} + N
\]  

(2)

where \( T_1 \) and \( T_2 \) are long and short relaxing components, respectively. The long \( (a_1) \) and short \( (a_2) \) fractions can be expressed in percentage as \( F_1 = 100 \times a_1/(a_1 + a_2) \) and \( F_2 = 100 \times a_2/(a_1 + a_2) \), respectively. To estimate the relaxation time constants and their fractions, the MR signal must be acquired at several time points. Under given signal to noise ratio (SNR), the smaller number of points is desired to minimize the scan time. To determine the adequate range and number of points for successful estimation, Mont Carlo simulation30 was performed for 1000 random noise trail with normal distribution \( N(0,\sigma) \). The SNR was defined as \( SNR = 1/\sigma \). The estimation errors were calculated for each noise trail as:

\[
E = \left| \frac{y_a - y_e}{y_e} \right|
\]  

(3)

where \( y_a \) and \( y_e \) are the actual and estimated values, respectively. The average of errors in 1000 trial was reported in percentage as the Monte Carlo simulation result.

In-vivo MRI acquisition. The study was approved by the institutional review board (IRB). All methods were performed in accordance with the relevant guidelines and regulations, and all of the participants signed a written informed consent prior to MRI scanning. Four females (age: 26 ± 3 years, BMI: 22 ± 1 kg/m²) and four male participants (age: 30 ± 3 years and BMI: 24 ± 2) with no signs of muscle pains or history of lower leg muscle injuries were recruited for this study. Additionally, follow-up scans were acquired from three participants two weeks after their first scan. 3D T1\( \rho \) and T2-weighted MR scans were taken on a 3T whole-body clinical MRI scanner (Prisma, Siemens Healthcare, Erlangen, Germany) with a 15-channel Tx/Rx knee coil (QED, Cleveland OH). 3D-Cartesian turbo-flash (TFL) sequence was used after T1\( \rho \) preparation module as readout followed by a T2 preparation module. The total scans time to acquire both T1\( \rho \) and T2-weighted data set was 29 min, 30 s. The sequence timing diagram is shown in Fig. 7. Fat-suppressed T1\( \rho \)- and T2-weighted scans were acquired in the sagittal plane at 10 different TSL/TEs including 2, 4, 6, 8, 10, 15, 25, 35, 45, and 55 ms (Fig. 2). The total scans time to acquire both T1\( \rho \) and the T2-weighted data set was 29 min, 30 s. The sequence acquisition parameters were as follows: TR/TE = 1500 ms/3.78 ms, flip angle = 8°, field of view (FOV) = 140 mm², matrix size 256 × 128 × 64, slice thickness = 2 mm, GRAPPA31 acceleration factor (AF) = 3. The spin-lock frequency (FSL) of 500 Hz was used in T1\( \rho \) preparation module.

Biexponential T1\( \rho \) and T2 relaxation mapping. The T1\( \rho \) and T2 data were analyzed using an in-house program developed in MATLAB (R2017a, The MathWorks Inc., Natick, MA, USA). The mono- and biexponential models were applied pixel by pixel over five consecutive slices for each volunteer in the five ROIs (Fig. 2): Gastrocnemius Medialis (GM), Gastrocnemius Lateralis (GL), Soleus (SOL), Peroneus longus (PER), and Tibialis Anterior (TA).

In the final biexponential estimation the pixels where \( 4T_{short} < T_{long} \) were excluded from the analysis27.

Statistical analysis. The statistical analysis was performed using JMP statistical software (JMP®, Version 13 SAS Institute Inc., Cary, NC, 1989–2007). Wilcoxon rank sum test was applied to compare T1\( \rho \) and T2 relaxation components as well as gender difference. T1\( \rho \) and T2 relaxation components were also compared in different ROIs using the Kruskal-Wallis test.

The repeatability studies were performed on three participants by repeating the scans after two weeks. The coefficient of variation (CV) for each participant was calculated as

\[
CV = \frac{\sigma}{\mu}
\]  

(4)

where \( \mu \) and \( \sigma \) are the mean and standard deviation of the estimated components from two scans, respectively. The root-mean-squared CV (RMSCV) was then calculated across three subjects to evaluate the inter-subject repeatability:

\[
RMSCV = \sqrt{\frac{\sigma_1^2 + \sigma_2^2 + \sigma_3^2}{\mu_1 + \mu_2 + \mu_3}}
\]  

(5)

Moreover, the intra-class correlation (ICC) was calculated as:
\[ \sigma^2 = \frac{ICC}{6} \]

where \( \sigma^2_b \) and \( \sigma^2_w \) are the between and within subjects variances, respectively.

**Data availability.** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions
All authors contributed to the conception and design of the study. A.S. performed the experiment, data analysis and interpretation of data. A.S. and R.R. drafted the article. G.C. and R.R. revised it critically for important intellectual content and approved the final version submitted. Dr. R.R. takes responsibility for the integrity of the work as a whole.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

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