Published in final edited form as:

NMR Biomed. 2015 August ; 28(8): 998–1008. doi:10.1002/nbm.3332.

Rapid Assessment of Quantitative T1, T2, and T2* in Lower Extremity Muscles in Response to Maximal Treadmill Exercise

Juliet Varghese^{1,2}, Debbie Scandling¹, Rohit Joshi¹, Ashish Aneja³, Jason Craft⁴, Subha V. Raman^{1,5}, Sanjay Rajagopalan⁶, Orlando P. Simonetti^{1,5,7}, and Georgeta Mihai^{7,8}

¹Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA

²Department of Biomedical Engineering, The Ohio State University, Columbus, OH, USA

³Department of Cardiology, MetroHealth Medical Center, Cleveland, OH, USA

⁴Department of Cardiology, Advocate Christ Medical Center, Oak Lawn, IL, USA

⁵Division of Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, USA

⁶Division of Cardiovascular Medicine, University of Maryland, Baltimore, MD, USA

⁷Department of Radiology, The Ohio State University Wexner Medical Center, Columbus, OH, USA

⁸Department of Radiology, Beth Israel Deaconess Medical Center, Boston, MA, USA

Abstract

MRI provides a non-invasive diagnostic platform to quantify the physical and physiological attributes of skeletal muscle at rest and in response to exercise. MR relaxation parameters (T1, T2 and T2*) are characteristic of tissue composition and metabolic properties. With the recent advent of quantitative techniques that allow rapid acquisition of T1, T2 and T2* maps, we posited that an integrated treadmill exercise-quantitative relaxometry paradigm can rapidly characterize exercise-induced changes in skeletal muscle relaxation parameters. Accordingly, we investigated the rest/recovery kinetics of T1, T2 and T2* in response to treadmill exercise in the anterior tibialis, soleus and gastrocnemius muscles of healthy volunteers, and the relationship of these parameters to age and gender. Thirty healthy volunteers (50.3 ± 16.6 years) performed the Bruce treadmill exercise protocol to maximal exhaustion. Relaxometric maps were sequentially acquired at baseline and for approximately 44 minutes post-exercise. Our results show that T1, T2 and T2* are significantly and differentially increased immediately post-exercise among the leg muscle groups, and these values recover to near baseline within 30–44 minutes. Our results demonstrate the potential to characterize the kinetics of relaxation parameters with quantitative mapping and upright exercise, providing normative values and some clarity on the impact of age and gender.

Corresponding Author: Orlando P. Simonetti, orlando.simonetti@osumc.edu, Physical address for correspondence: 320 BRT, 460 W. 12th Avenue, Columbus, OH 43210, USA.

DISCLOSURES

SVR and OPS have financial interests in EXCMR, Inc. SVR and OPS receive research support from Siemens Healthcare, Inc.

Graphical abstract

Quantitative T1, T2 and T2* maps of lower extremity skeletal muscles were sequentially acquired at rest and post-treadmill exercise in an integrated examination, to non-invasively evaluate rest/recovery kinetics. Treadmill exercise caused differential increase in relaxometric measures in muscle groups, which subsequently recovered to resting values and were influenced by age and gender. Quantitative T1, T2 and T2* may provide rapid non-invasive measures of pathophysiology and functional impairment in characterizing disease severity.

Keywords

magnetic resonance relaxometry; treadmill exercise; calf muscle; aging

INTRODUCTION

The current approach to management of circulatory disorders such as peripheral arterial disease (PAD) is to focus on the detection and treatment of stenoses in large conduit vessels of the lower extremity. There is a compelling need to develop complementary techniques to provide better insights into the pathophysiology of functional impairment resulting from the effects of PAD on the end organ: the lower extremity skeletal muscle. MRI provides an excellent non-invasive diagnostic platform to quantify the physical and physiological attributes of skeletal muscle at rest and in response to interventions such as exercise (1).

While T1, T2 and T2* exhibit a consistent range within normal muscles that is characteristic of tissue composition and metabolic properties, the pathophysiological basis of abnormal changes continues to be debated (2, 3). Changes in T1, T2, and T2* with muscle activation have also been evaluated, with the hope that these surrogates may provide insights into pathophysiological states associated with skeletal muscle injury and disease such as PAD (4, 5). In general, however, most MR studies of relaxation parameters have been limited to the investigation of a single relaxometric parameter at a time, typically in response to

interventions that may not be analogous to upright, weight-bearing exercise that reflects normal daily activity and is routinely used to assess the impact of PAD on skeletal muscle.

The exact mechanisms for exercise-induced changes in T1 of skeletal muscles have not been studied in great detail to date mostly due to the long imaging times necessary for T1 quantification. The genesis of T1 changes has been attributed to increased blood volume in the vascular compartment, osmotic fluid shifts and temperature (4, 6, 7). Increase in T2 following electrical stimulation was first demonstrated in the amphibian muscle (8). Exercise-dependent changes in protein concentration and pH drive osmotic shifts of muscle water that increase the volume of intracellular space (accumulation of lactate, phosphate, and sodium causes an influx of fluid); intracellular acidification (9, 10) that results from the end products of metabolism also slows the decay of transverse relaxation, thereby increasing T2. As such, exercise-induced changes in T2 are related to the metabolic activity of the muscle cells (11).

Skeletal muscle Blood Oxygen Level Dependent (BOLD) contrast results from changes in the microvascular ratio of oxyhemoglobin to deoxyhemoglobin, and reflects tissue oxygenation and oxygen extraction at the microvascular level (5, 12). It is considered an index of muscle perfusion and peripheral vascular function (13). The BOLD effect can be studied by exercise-provoked alterations in quantitative T2* maps. The BOLD signal, which is dependent on T2*, increases with exercise due to increased muscle perfusion, followed by subsequent decrease to baseline with recovery (12).

To the best of our knowledge, to date there has been no study in which skeletal muscle T1, T2, and T2* have been assessed before and after upright treadmill exercise to exhaustion. With the advent of rapid quantitative mapping techniques designed for cardiac imaging, we hypothesize that the physiological responses of the skeletal muscle at rest and with exercise can be quantified by rapid sequential acquisition of T1, T2 and T2* maps with a comprehensive treadmill exercise-imaging protocol. The combination of treadmill testing plus skeletal muscle relaxometry could potentially be used to rapidly characterize and understand pathophysiologic alterations in the skeletal muscle that accompany diseases such as PAD. In the present study, we tested the above-stated hypothesis by investigating the feasibility of quantitative MR relaxometry (T1, T2 and T2*) of the anterior tibialis, soleus and gastrocnemius muscles at rest and following maximal treadmill exercise, and by exploring their relationship to age and gender.

METHODS

Subject population

Thirty healthy volunteers ranging in age from 24 to 79 years (50.3 ± 16.6 years, 15 males) were subjected to treadmill exercise testing combined with quantitative MR relaxometric mapping of lower leg muscles. All volunteers had no MR contraindications and were free of cardiovascular disease and injury to the lower extremities. The volunteers were asked to not perform any strenuous exercise on the day of the study. Additionally, one patient with documented PAD (male, age 73 years) was tested to demonstrate the clinical potential for the combined exercise-relaxometry protocol.

MRI imaging system

All volunteers were imaged using a 1.5T whole body MRI system (MAGNETOM, Avanto, Siemens Healthcare, Erlangen, Germany) with maximum gradient amplitude of 45 mT/m and maximum slew rate of 200 mT/m/ms. The system's integrated body coil was used for signal transmission. A flexible six-element phased array body coil placed over the lower legs, combined with six corresponding elements of the 24-element spine coil embedded in the table, was used for signal reception.

MRI-compatible treadmill

An MRI-compatible treadmill (EXCMR, Columbus, OH, USA), previously developed by our laboratory (14), was positioned adjacent to the MR table (Figure 1A). The MR-safe treadmill is built of non-ferromagnetic components and remotely run by a hydraulic power system.

Subject preparation

The study was approved by the University's Institutional Review Board and was HIPAA compliant. After written informed consent and MRI screening, the volunteer was positioned on the MR table, feet-first into the scanner, by means of two vacuum cushions placed beneath the head/shoulders and the lower extremities; the lower cushion material was arranged so as to minimize the distance between the lower leg and the coils. Using an in-room vacuum line, the cushions were deflated to shape tightly around the volunteer, creating a permanent mold for the volunteer to get back into the pre-exercise position after completion of the treadmill exercise test. This arrangement ensured that pre- and post-exercise slice positioning was comparable without the need for additional localizer images, enabling the resumption of post-exercise imaging with minimum delay. Each subject's electrocardiogram (ECG) was monitored during the treadmill exercise test using a 12-lead ECG monitor (Cardiosoft, GE Healthcare, UK); brachial blood pressure was monitored using an MRI-compatible sphygmomanometer; heart rate was monitored during imaging with an MRI-compatible, three-lead wireless device.

MRI protocol

Single-shot, balanced steady-state free precession (SSFP) scout images were initially acquired for slice positioning. A single axial slice was defined at the level of the mid-calves (maximum girth visually) for all subsequent image acquisitions. A high resolution T1-weighted turbo spin echo (T1w-TSE) image ($TR/TE = 550/17$ ms, one average (number of excitations NEX), $0.7 \times 0.7 \times 5$ mm³, acquisition time $TA = 2$ min 25 s) was acquired to identify the leg muscle groups. At the same location, single-slice T1 maps were acquired using the Modified Look-Locker Imaging (MOLLI) technique (15) ($TR/TE/TI = 1200/1.04/125, 205, 285, 1325, 1405, 1485, 2525, 2605, 2685, 3885$ ms, flip angle, $FA = 35^\circ$, NEX = 1, $1.9 \times 1.9 \times 8$ mm³, $TA = 1$ min 2 s) with fat suppression, T2 maps using a technique based on T2-prepared SSFP (16) ($TR/TE_{T2p} = 2500/0, 24, 36, 48, 60$ ms, $FA = 70^\circ$, NEX = 2, $1.9 \times 1.9 \times 8$ mm³, $TA = 50$ s), and T2* maps using a multi-echo, gradient recalled echo technique (17) ($TR/TE_{1-8} = 740/2.1, 4.2, 6.6, 8.9, 11.3, 13.7, 16.1, 18.4$ ms, $FA = 18^\circ$, NEX = 2, $1.9 \times 1.9 \times 8$ mm³, $TA = 31$ s). These parameter maps were sequentially

acquired at rest over a period of about 15 min (six acquisition time points of T1, T2 and T2*) to ensure that the muscles had reached a physiological steady state represented by no significant temporal variation in relaxation parameters. All relaxometric maps were acquired with a field of view of 360 mm × 180 mm to include axial cross sections of both the left and right legs. The volunteers breathed freely and no cardiac triggering was used. After acquiring images at rest, the table was slid out of the scanner; resting ECG and blood pressure measurements were then recorded with the subject supine as well as standing on the treadmill. The subject then commenced the Bruce treadmill exercise protocol (starting at 1.7 mph and 10 % gradient, 3 min stages with increasing speeds of 2.5, 3.4, 4.2, 5.0, 5.5 and 6.0 mph, and 2 % increments in gradient), which was continued until maximum exhaustion (18). All volunteers performed treadmill exercise under the supervision of a cardiologist or an exercise physiologist. At the cessation of exercise, the volunteer was immediately repositioned on the MR table, and imaging was resumed. The time between the cessation of exercise and the start of post-exercise imaging was recorded. Post-exercise imaging involved 18 sets of sequential acquisitions of the relaxometric maps (T1, T2 and T2*) at the same pre-exercise slice location at intervals of 2 min 23 s; the total duration of post-exercise imaging was approximately 44 min. The imaging protocol is diagrammed in Figure 1B. In addition, the one patient with PAD exercised to the Skinner-Gardner protocol (constant speed of 2 mph, and 2 min increments in gradient by 2%) and underwent the same imaging protocol as the volunteers.

Fat fraction measurements

In 17 of the volunteers (nine male), fat-water images were acquired prior to exercise using the VARPRO (variable-projection-based) fat-water separation method (19) with TR/TE₁₋₄ = 86.31/1.37, 3.52, 5.67, 7.82 ms, FA = 24⁰, NEX = 32, 1.1×1.1×8 mm³, and TA = 50 s. A low FA was used to minimize the effects of T1. A pixel-wise fat fraction map was generated as fat/(fat+water) using the corresponding fat and water images for each volunteer in Matlab (The Mathworks, Inc, Natick, MA, USA) (20) (Figure 2B). A fat fraction for each of the three investigated muscles was defined as the average value within each muscle, averaged across both legs.

Test-retest reproducibility

Seven of the volunteers returned within an interval of 2–5 days for a repeat study. Treadmill exercise was performed identically to their first test (following the Bruce protocol for the same duration and distance); imaging slice location was matched by measuring the distance from the knee to the axial slice location of the first test, as well as visual identification of anatomical features in the images.

Quantitative measurements

The T1w-TSE image and all relaxometric maps were loaded into ImageJ (National Institutes of Health, Bethesda, MD, USA) for evaluation. The resting muscle cross-sectional area was evaluated by tracing the contour of the muscles bilaterally, excluding subcutaneous fat and including all muscle groups, on the high resolution T1w-TSE image (Figure 2A). For each volunteer, the anterior tibialis, soleus, and gastrocnemius muscles of the right and left legs were manually segmented, avoiding large blood vessels and fasciae (Figure 2A). The

contours were then copied onto all T1, T2 and T2* maps. The median pixel values from each of the three muscle groups for both legs were recorded. The right and left leg measurements for each muscle were averaged together in each volunteer at each of the 24 measured time points. The resting value (baseline), peak value after exercise (peak) and final value at the end of recovery post-exercise (recovery) were recorded for T1, T2 and T2*. The rate of decay (τ) was determined by mono-exponentially fitting the post-exercise measurements to the equation,

$$S(t) = a + b * \exp(-t/\tau) \quad (1)$$

where $S(t)$ is the measured parameter (T1, T2, T2*) at each time point t , after exercise.

Statistical analysis

Statistical analysis was performed using SPSS (Version 21.0: IBM, Armonk, NY, USA). All continuous variables are presented as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used to evaluate normality of the measured variables; non-parametric tests were used for group analyses as some of the data were not normally distributed. The non-parametric Wilcoxon signed rank test was used to determine if the post-exercise peak and recovery relaxometric values were significantly different from baseline, and to analyze differences between the three muscle groups. Linear regression was used to explore the relationship of the relaxometric measures with age and gender, adjusted for the effects of treadmill test duration for post-exercise measures. A Bonferroni-corrected probability (p) value of less than 0.017 was considered to be statistically significant for these analyses. Correlations were also examined among age, treadmill test duration and fat fraction ($p < 0.05$ considered significant). On the subjects undergoing repeat tests, the reproducibility of baseline, peak and recovery T1, T2 and T2* measures for the three muscle groups were assessed using the coefficient of variation (CV) and intraclass correlation coefficient (ICC - calculated using a two way mixed effects model).

RESULTS

Thirty volunteers were recruited and all successfully completed the exercise-imaging protocol. Table 1 shows the mean age, body mass index (BMI), muscle cross-sectional area, treadmill test duration, blood pressure and heart rates for the cohort.

Exercise response in leg muscles

The first post-exercise map was acquired after exercise cessation within a range of 25–73 s (39.9 ± 11.4 s) in all volunteers. Figure 3 shows example images of the relaxometric changes seen immediately after treadmill exercise in the lower right leg of one volunteer. The percentage change from baseline and the average value at each time point for all volunteers are shown in Figure 4(A–C), providing an index of the typical treadmill exercise-induced response in healthy leg muscles. The mean $\pm$ SD of the T1, T2, and T2* values at baseline, peak, and recovery for all volunteers, with significant post exercise increase from baseline, are shown in Figure 4(D–F). The differences between baseline and recovery values were generally not significant, although soleus T1 at the end of imaging showed slightly

elevated values compared with baseline in 24 out of 30 subjects. The exponential rate of decay from post-exercise peak of T1, T2 and T2* varied widely, from 3 min to 1 h; generally, longer rates of decay were seen in T1 than in T2. The mean values from all muscle groups and significant differences among them are summarized in Table 2.

Associations with age

Significant correlations were observed with age after adjusting for the effects of treadmill test duration. The p values are shown in Table 3. T1 baseline values in the anterior tibialis and gastrocnemius muscles were higher, while the post-exercise T1 increase in the gastrocnemius muscle was inversely correlated with age. Baseline, peak and recovery T2 values were significantly higher with age in the soleus muscle, while baseline and recovery T2 positively correlated with age in the gastrocnemius muscle. T2* showed a decline with age in the soleus muscle at baseline, peak and recovery.

Muscle cross-sectional area and fat fraction

Muscle cross-sectional area did not show any significant relationship with age. Fat fraction was found to increase in all muscle groups with age (anterior tibialis, $R = 0.49$, $p = 0.046$; soleus, $R = 0.79$, $p < 0.001$; gastrocnemius, $R = 0.68$, $p = 0.003$), and ranged from a low of 2.6% in the anterior tibialis of one young volunteer (30 years), to a high of 15.8% in the soleus of one older volunteer (73 years). The mean values are shown in Table 2. Treadmill test duration correlated negatively with the fat fraction of gastrocnemius ($R = -0.61$, $p = 0.01$), but not anterior tibialis ($R = -0.25$, $p = 0.33$) or soleus ($R = -0.41$, $p = 0.11$) (Figure 5).

Associations with gender

After adjusting for exercise duration, women showed longer T1 at peak and recovery in the anterior tibialis muscle, and at baseline, peak and recovery in the soleus muscle (Table 3). T2* was longer in women at recovery in the anterior tibialis and at baseline and recovery in the soleus. Higher post-exercise % T1 in the gastrocnemius muscle was seen in men. Females had significantly smaller muscle cross-sectional area than males (6787.0 ± 1097.2 vs 8162.6 ± 1088.9 mm², $p = 0.007$).

Test-retest reproducibility

Table 4 shows the mean and SD of relaxometric measures from the first and second examinations, along with CV and ICC. CV ranged from 1.1 to 3.6, while ICC ranged from 0.4 to 0.9.

PAD Patient

The relaxometric maps and response from the PAD patient are shown in Figure 6. The subject performed treadmill exercise for a duration of 5 min 6 s. Although the exercise was primarily stopped due to shortness of breath, the patient also reported claudication. The resting muscle cross-sectional areas of the right and left calves were 7245.8 and 7262.4 mm² respectively. The right and left fat fractions were 14.0% and 13.6% in anterior tibialis, 18.0% and 18.9% in soleus and 14.8% and 14.2% in the right and left gastrocnemius

muscles respectively, and were greater than two SDs higher than the mean measured in volunteers.

DISCUSSION

This study investigated the feasibility of an integrated quantitative MR relaxometry-treadmill exercise paradigm, and explored the influence of age and gender on T1, T2 and T2* relaxation times in the major lower leg muscle groups at rest and immediately following maximal treadmill exercise. This is the first study to dynamically monitor and quantify changes in all relaxometric parameters after upright treadmill exercise in a clinical setting, demonstrating a protocol that could potentially be used to evaluate the effects of PAD. The main findings of this study were that (i) T1, T2 and T2* increase following treadmill exercise and recover to near baseline after 30–44 min, (ii) the responses of T1, T2, and T2* to exercise differ between the muscle groups, (iii) older age is associated with higher baseline T1 and T2 values in these muscle groups, (iv) fat content increases with age and (v) T1 of the anterior tibialis and soleus tends to be longer in women.

Our findings regarding differences in the baseline resting relaxation times among the investigated leg muscle groups agree with previous studies that have attributed these variations to muscle composition (20, 21). Although we averaged the bilateral relaxometric measurements (the muscles of both legs will most likely behave similarly in healthy volunteers), images including both legs were acquired. This was to account for the possibility that this protocol could be utilized to evaluate bilateral disease severity in PAD patients in the future.

We saw the greatest post-exercise T1, T2 and T2* increase in the gastrocnemius, followed by anterior tibialis and soleus muscles. These findings are consistent with the metabolic predisposition and functions of the muscle groups. Soleus and gastrocnemius muscles differ in their fiber type (slow vs fast twitch), mitochondrial content (greater in soleus), glycogen capacity (greater in gastrocnemius) and oxidative phosphorylation (greater in soleus) (22).

The study of Fleckenstein et al. was the first to demonstrate that MRI could differentiate exercise-induced muscle activation by means of increases in T1 and T2 relaxation times, which they showed persisted for more than 10 min post-exercise (4). The accumulation of water in normally perfused exercising muscles as a consequence of intense shifts in osmotically active elements has been postulated to underlie the changes in T1 and T2, and has been reported extensively (9, 11, 23). These studies have clearly shown that T1 and T2 increase by 10–30% in normal muscles in response to exercise, and that T2 recovers back to its resting level within 20–35 min (24, 25). While their exercise methods involved plantar flexion and toe extension exercises, the present study achieved similar results with dynamic, upright treadmill exercise and by using modern, rapid quantitative parameter mapping techniques.

It is known that changes in fiber composition and fat infiltration occur with age (26, 27); these differences could be reflected in the increased baseline relaxometric values and increased fat fraction seen in our study. While a relationship between T1 of resting skeletal

muscle and age has not been previously demonstrated, T1 of skeletal muscle has been shown to increase following denervation (28, 29); this has implications for an older patient population, many of whom may have subclinical neural abnormalities. T1 has also been shown to correlate with the percentage of type I fibers, which can increase in muscles of older individuals, and which have a greater extracellular volume fraction than other fiber types (30, 31). Previous studies have shown an age-related T2 increase in lower leg muscles (21, 26). They postulated the cause as increased extracellular space and accumulation of extracellular fluid in atrophied muscles. While skeletal muscle BOLD response has mostly been studied with single-contraction models and cuff occlusion, our dynamic exercise paradigm showed an increase in T2* that may be attributed to the BOLD effect. Fatty infiltration may account for some of the increase in T2 seen with aging and may present an important confounder. Increased muscle lipid deposition has been postulated as a mediator of skeletal muscle insulin resistance that occurs with aging and may need to be factored in when assessing baseline T2 values and/or response to exercise. While acquisition of fat suppressed T2 and T2* images would remove the confounding effects of fat on T2 and T2* measurements, this was not done in the interest of image quality, which was found to be superior when fat suppression was not used in these maps.

The influence of age on post-exercise relaxometric measures after accounting for exercise performance may possibly suggest loss of muscle mass and atrophy of type II fibers in older individuals, especially in the gastrocnemius muscle. Although the overall muscle cross-sectional area did not show any reduction with age, it is likely that intramuscular fat replaced muscle volume, as evidenced by the increased fat fraction we observed with age. The exercise times varied among individuals; however, considering future applications in PAD patients who are clinically tested for maximum walking distance as a measure of disease severity, we believe that a maximal treadmill test provided an adequate comparison in healthy volunteers.

Regarding the influence of gender, our results showed that females had smaller cross-sectional area, and longer T1 and T2* in the anterior tibialis and soleus muscles. A recent MRI study by Piechnik et al. (32) found T1 of the myocardium and blood to be longer in females. A likely cause for the longer T1 in skeletal muscles in women could be greater extracellular fluid space, due to smaller muscle mass, smaller fiber size and smaller volume fraction devoted to fibers compared with men (33). There are also known gender differences in motor neuron activation, muscle morphology and substrate utilization. In addition, females have higher resting blood flow relative to muscle mass, and lower oxygen extraction rate but the same oxygen consumption as males (34). These conditions may influence tissue oxygenation levels, and possibly contribute to longer T2*. These findings might have implications in a patient population, as functional decline and mobility loss with PAD is found to be exacerbated in women (35).

One PAD patient was examined to demonstrate the feasibility of the proposed exercise-MRI protocol. The right leg of the patient had undergone bypass surgery while the left leg remained affected by disease. The T1 and T2 responses observed in the soleus muscle differed between the two limbs. T2 of the left soleus showed higher baseline, possibly indicative of edema, while baseline soleus T2* of both legs was lower than in healthy

volunteers. T2 of left soleus did not show an increase after exercise. Thus, while this is only a single, anecdotal example, it highlights the marked differences in relaxometric responses that may be encountered in patients with PAD.

The assessment of post-exercise changes in T1, T2 and T2* and their physiologic determinants may provide important information where there is currently a significant lacuna of understanding. Although other MRI-compatible exercise equipment and interventions have been successfully used in lower extremity MRI studies, only an upright treadmill provides a true reproduction and assessment of the physiologic and metabolic responses involved in walking, and in addition replicates the graded treadmill exam commonly used to clinically evaluate PAD patients. The results of other test protocols may be more limited: for example, muscle recruitment and activation during supine plantar flexion is influenced by the degree of knee joint flexion, and cuff occlusion may mostly affect the soleus muscle without gastrocnemius involvement.

There were limitations in this study that was primarily designed to evaluate feasibility and establish normal values and age dependency of rapid quantitative relaxometric maps acquired immediately after treadmill exercise. One important limitation was the mixing of trained and untrained individuals in the cohort. Muscular and vascular adaptation with routine exercise training may have influenced the overall relaxometric response in some individuals in this study.

The present study did not compare relaxometry against biopsy or histopathology, so the proposed mechanisms behind our observations are based on the physiological factors known to affect relaxation parameters. In addition, three volunteers above 70 years of age were on blood pressure medications, and one volunteer on thyroid medication. However, the relaxometric responses in these individuals did not vary significantly from the others. It is also possible that the gap in time between exercise cessation and post-exercise imaging may have influenced our results. Phosphocreatine (PCr), inorganic phosphate (Pi) and adenosine triphosphate recovery kinetics post-exercise are rapid (105 – 135 s), making it difficult to characterize these responses without in-magnet exercise. Although T2 has been shown to correlate with Pi/PCr measures (36), in this study the first post-exercise T2 map was typically acquired in the second or third minute after exercise cessation; therefore, it may be that we were unable to capture rapid metabolic changes occurring immediately post-exercise. Nevertheless, as relaxation parameters recover to baseline values over the course of several minutes, we believe that the gap in time between end of exercise and acquisition of the first parameter maps should not greatly affect the overall measured relaxometric response.

Another limitation of our study was the imaging of only a single cross-sectional slice. The non-selective magnetization preparation schemes employed in the rapid MRI quantification techniques used in this study preclude the rapid acquisition of multiple slices; therefore, single-slice acquisitions were used in the interest of temporal resolution to track dynamic changes in relaxation parameters after cessation of exercise. However, there is potential for future studies with multi-slice acquisitions, as shown recently with myocardial T1 mapping (37).

The Bruce treadmill exercise protocol is an established measure of cardiorespiratory fitness; volunteers may have stopped treadmill exercise primarily due to cardiovascular exhaustion rather than leg muscle pain or fatigue. However, all subjects reported skeletal muscle fatigue concomitant with cardiovascular exhaustion, and we measured increased relaxation times in all muscle groups. Thus, we believe that the intent of this study to develop a protocol that would set the normal standards for future tests in patients who are likely to be limited in exercise duration due to claudication was achieved.

In conclusion, we have demonstrated the feasibility of rapid quantification of multiple MR relaxation parameters (T1, T2 and T2*) in skeletal muscle at rest, and in response to treadmill exercise in healthy subjects within a single examination, with the intent that these data will help subsequent studies geared towards understanding exercise adaptation in patients with PAD, and eventually in the design of interventional therapeutic studies in PAD. This study is the first to show the near simultaneous behavior and inter-relatedness of all three relaxation times in the skeletal muscle at rest and post-exercise. While each relaxometric measure is a useful physiological and pathological index - T1 measures may provide a measure of exercise-induced muscle activation, and T2 and T2* may be sensitive to pathological tissue changes and functional impairment – combined information from all three may create a better picture regarding skeletal muscle function. MR relaxometric measures may thus serve as relevant surrogates to provide deeper insight into exercise physiology, in understanding the pathophysiology of diseases that affect skeletal muscle integrity, and in monitoring the progressive effects of exercise training and rehabilitation activities that aim to improve functional capacity.

Acknowledgments

We thank Olivia Latkovic and Bradley Thornburg for their supervision of subjects during treadmill exercise.

GRANTS

This study was supported by American Heart Association pre-doctoral fellowship AHA 13PRE16950001 (JV), by award number R01HL102450 from the National Heart, Lung, And Blood Institute (OPS and SVR), and the OSUMCCOM Pilot Grant from The Ohio State University (GM).

ABBREVIATIONS USED

MRI	Magnetic Resonance Imaging
PAD	Peripheral Arterial Disease
BOLD	Blood Oxygen Level Dependent
HIPAA	Health Insurance Portability and Accountability Act
ECG	Electrocardiogram
SSFP	Steady-State Free Precession
T1w	T1-weighted
TSE	Turbo Spin Echo

TR	Repetition Time
TE	Echo Time
NEX	Number of Excitations
TA	Acquisition Time
MOLLI	Modified Look–Locker Imaging
FA	Flip Angle
SD	Standard Deviation
BMI	Body Mass Index
CV	Coefficient of Variation
ICC	Intra Class Correlation Coefficient
PCr	Phospho Creatine

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Figure 1.

(A) Setup of MR-compatible treadmill adjacent to the MR scanner. (B) Time course of imaging-exercise protocol and characterization of exercise dynamics – imaging at rest (Baseline), peak exercise (Peak), % increase, end of recovery values (Recovery) and post-exercise recovery time constant (τ). The time points of imaging at rest (B1–B6) and after exercise (P1–P18) are depicted.

Figure 2.

(A) High resolution T1w-TSE axial image showing calf muscle groups of the left leg. The shaded areas were selected to generate the relaxation times for the investigated muscle groups: anterior tibialis muscle (TA), soleus muscle (Sol), and gastrocnemius muscle (Ga). The dotted contour shows the region for determining muscle cross-sectional area. (B) A corresponding fat fraction map is shown on the right.

Figure 3.

Example of the relaxometric maps of the right calf from a young healthy subject, depicting the time course of exercise induced changes. Axial T1 (top row), T2 (middle row) and T2* (bottom row) maps acquired before exercise (Baseline), immediately after exercise (Peak), and about 42 min later (Recovery) are shown. The time after exercise at which the images were acquired is indicated within the box. Increased relaxation times are seen in the Peak maps (clearly evident in the gastrocnemius muscle) followed by a recovery to resting state.

Figure 4.

Exercise-induced time course changes of %T1 (A), %T2 (B) and %T2* (C) in volunteers for anterior tibialis, soleus and gastrocnemius muscles. The mean $\pm$ SD of T1 (D), T2 (E) and T2* (F) at baseline, peak and recovery for the three muscle groups are shown.

*Significant differences from baseline determined by Wilcoxon signed rank test, $p < 0.017$

Figure 5.
Relationship of % fat in anterior tibialis (TA) (A), soleus (B) and gastrocnemius ($p < 0.05$) (C) muscles to treadmill exercise duration.

Figure 6. Relaxometric maps of bilateral calves at baseline, peak and end of post-exercise recovery in a PAD patient. The corresponding relaxometric responses for the three muscle groups in the right and left calves are shown below.

Table 1Demographic and physiologic characteristics of the volunteers (mean $\pm$ SD) N = 30

Age, years	50.3 $\pm$ 16.6
Gender	15 males
BMI, kg/m ²	25.2 $\pm$ 3.7
Muscle CSA, mm ²	7474.8 $\pm$ 1281.8
Treadmill test duration, min	11.0 $\pm$ 3.2
Rest SBP, mm Hg	120.5 $\pm$ 14.4*
Rest DBP, mm Hg	76.7 $\pm$ 7.7*
Max recorded SBP, mm Hg	165.4 $\pm$ 15.3 [†]
Max recorded DBP, mm Hg	74.3 $\pm$ 7.7 [†]
Resting heart rate, bpm	74.0 $\pm$ 10.9
Peak heart rate, bpm	162.9 $\pm$ 17.6
APMHR, %	98.3 $\pm$ 14.7

CSA, cross-sectional area; SBP, systolic blood pressure; DBP, diastolic blood pressure; APMHR, age-predicted maximum heart rate.

* N = 20,

[†] N = 12

Table 2

T1, T2 and T2* characteristics of all volunteers in anterior tibialis, soleus and gastrocnemius muscles (mean $\pm$ SD)

	Anterior tibialis	Soleus	Gastrocnemius
T1			
Baseline, ms	815.7 $\pm$ 26.8	827.7 $\pm$ 29.0 *	861.8 $\pm$ 57.8 **
Peak, ms	891.9 $\pm$ 29.9	898.1 $\pm$ 27.1	960.1 $\pm$ 49.7 **
Recovery, ms	818.9 $\pm$ 23.3	835.9 $\pm$ 24.3 *	866.3 $\pm$ 46.3 **
% increase	9.4 $\pm$ 3.4	8.5 $\pm$ 1.9	11.5 $\pm$ 3.5
τ , min	13.9 $\pm$ 4.2	12.0 $\pm$ 3.3	23.8 $\pm$ 9.9
T2			
Baseline, ms	35.9 $\pm$ 1.5	38.3 $\pm$ 2.0 *	40.4 $\pm$ 3.7 **
Peak, ms	39.7 $\pm$ 2.3	41.4 $\pm$ 1.8 *	46.0 $\pm$ 3.9 **
Recovery, ms	35.8 $\pm$ 1.7	38.4 $\pm$ 2.2 *	39.9 $\pm$ 3.5 **
% increase	10.8 $\pm$ 6.1	8.9 $\pm$ 3.7	14.2 $\pm$ 6.4
τ , min	12.2 $\pm$ 6.3	15.1 $\pm$ 10.4	14.3 $\pm$ 7.0
T2*			
Baseline, ms	27.6 $\pm$ 1.1	27.3 $\pm$ 2.3	29.3 $\pm$ 1.7 **
Peak, ms	31.4 $\pm$ 1.9	30.7 $\pm$ 2.8	35.1 $\pm$ 2.9 **
Recovery, ms	27.3 $\pm$ 1.2	27.5 $\pm$ 2.1	29.1 $\pm$ 1.6 **
% increase	14.1 $\pm$ 7.5	13.9 $\pm$ 3.9	20.0 $\pm$ 7.6
τ , min	14.2 $\pm$ 9.8	27.5 $\pm$ 26.2	15.4 $\pm$ 13.8
Fat fraction, % (n = 17)	4.9 $\pm$ 2.4	7.0 $\pm$ 3.2	5.5 $\pm$ 2.0

* Significantly higher than anterior tibialis muscle (p < 0.017)

** Significantly higher than anterior tibialis and soleus muscles (p < 0.017)

Table 3
Effect of age and gender on relaxometric measures - post-exercise p values adjusted for treadmill duration

	Age			Gender		
	Anterior Tibialis	Soleus	Gastrocnemius	Anterior Tibialis	Soleus	Gastrocnemius
T1						
Baseline, ms	0.006*	0.038	0.012*	0.018	<0.001*	0.203
Peak, ms	0.743	0.023	0.287	0.015*	0.001*	0.507
Recovery, ms	0.163	0.071	0.048	0.001*	0.001*	0.121
% increase	0.054	0.907	0.003*	0.042	0.11	0.004*
τ , min	0.086	0.052	0.3	0.164	0.095	0.164
T2						
Baseline, ms	0.034	0.002*	0.004*	0.861	0.555	0.685
Peak, ms	0.182	0.01*	0.196	0.499	0.046	0.621
Recovery, ms	0.046	0.002*	0.014*	0.991	0.433	0.292
% increase	0.424	0.3	0.077	0.512	0.122	0.031
τ , min	0.637	0.272	0.704	0.487	0.457	0.659
T2*						
Baseline, ms	0.499	0.003*	0.502	0.051	0.010*	0.107
Peak, ms	0.627	0.006*	0.958	0.329	0.267	0.905
Recovery, ms	0.485	0.006*	0.419	0.002*	0.005*	0.183
% increase	0.62	0.369	0.573	0.668	0.092	0.085
τ , min	0.65	0.395	0.498	0.534	0.985	0.341

* p < 0.017 significant

Table 4

Test-retest reproducibility of baseline, peak and recovery values (mean $\pm$ SD) from the two visits

	Baseline			Peak			Recovery		
	Visits 1 and 2	CV	ICC	Visits 1 and 2	CV	ICC	Visits 1 and 2	CV	ICC
T1									
Anterior tibialis	800.6 $\pm$ 17.8	1.2	0.8	891.4 $\pm$ 35.1	1.1	0.9	810.8 $\pm$ 20.8	1.3	0.8
	806.1 $\pm$ 21.7			893.8 $\pm$ 43.6			815.9 $\pm$ 26.3		
Soleus	813.6 $\pm$ 27.6	1.5	0.9	881.1 $\pm$ 32.9	1.2	0.9	828.2 $\pm$ 24.4	1.6	0.8
	816.5 $\pm$ 38.9			882.2 $\pm$ 42.6			827.4 $\pm$ 33.9		
Gastrocnemius	811.7 $\pm$ 27.1	2.8	0.6	928.1 $\pm$ 28.4	2.0	0.6	832.5 $\pm$ 25.8	1.6	0.8
	816.6 $\pm$ 45.2			924.1 $\pm$ 36.1			827.6 $\pm$ 37.8		
T2									
Anterior tibialis	35.9 $\pm$ 1.4	1.3	0.9	40.2 $\pm$ 2.2	2.8	0.9	36.0 $\pm$ 1.6	2.8	0.8
	35.6 $\pm$ 1.3			38.9 $\pm$ 2.4			35.1 $\pm$ 1.8		
Soleus	37.9 $\pm$ 1.4	2.1	0.7	40.3 $\pm$ 1.7	1.8	0.9	38.2 $\pm$ 1.5	2.7	0.4
	37.6 $\pm$ 1.7			39.7 $\pm$ 1.7			37.8 $\pm$ 1.1		
Gastrocnemius	38.2 $\pm$ 1.6	1.8	0.9	44.6 $\pm$ 2.7	3.4	0.7	38.3 $\pm$ 2.4	3.4	0.5
	37.8 $\pm$ 2.3			43.4 $\pm$ 1.8			37.7 $\pm$ 1.1		
T2*									
Anterior tibialis	27.6 $\pm$ 1.5	2.1	0.9	31.3 $\pm$ 2	3.6	0.8	27.5 $\pm$ 1.6	2.9	0.9
	27.4 $\pm$ 1.7			30.7 $\pm$ 2.5			27.0 $\pm$ 2.2		
Soleus	28.3 $\pm$ 1.6	1.7	0.9	30.6 $\pm$ 2.1	2.9	0.8	28.7 $\pm$ 1.8	2.3	0.9
	28.3 $\pm$ 1.8			30.8 $\pm$ 2.5			28.3 $\pm$ 2.1		
Gastrocnemius	28.7 $\pm$ 1.0	2.7	0.6	34.4 $\pm$ 2.5	2.4	0.8	28.7 $\pm$ 1.4	1.3	0.9
	28.6 $\pm$ 1.6			34.4 $\pm$ 1.9			28.4 $\pm$ 1.4		

CV, Coefficient of Variation; ICC, Intraclass Correlation Coefficient.