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Diffusion weighted MRI with intravoxel incoherent motion modeling for assessment of muscle perfusion in the thigh during post-exercise hyperemia in younger and older adults

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Abstract

Aging is associated with impaired endothelium-dependent vasodilation that leads to muscle perfusion impairment and contributes to organ dysfunction. Impaired muscle perfusion may result in inadequate delivery of oxygen and nutrients during and after muscle contraction leading to muscle damage. The ability to study the relationship between perfusion and muscle damage has been limited using traditional muscle perfusion measures, which are invasive and risky. To overcome this limitation, we optimized a diffusion-weighted MRI sequence and validated an intravoxel incoherent motion (IVIM) analysis based on Monte Carlo simulation to study muscle perfusion impairment with aging during post-exercise hyperemia. Simulation results demonstrated that the bias of IVIM-derived perfusion fraction (f_p) and diffusion of water molecules in extra-vasculature tissue (D) ranged from -3.3% to 14% and from -16.5% to 0.002%, respectively, in the optimized experimental condition. The dispersion in f_p and D ranged from 3.2% to 9.5% and from 0.9% to 1.1%, respectively. The mid-thigh of the left leg of four younger (21-30 year-old) and four older (60-90 year-old) healthy females was studied using the optimized protocol at baseline and at 7 time increments occurring every 3.25min following in-magnet dynamic knee extension exercise performed using a MR-compatible ergometer with a workload of 0.4bar for 2.5min. After exercise, both f_p and D significantly increased in the rectus femoris (active-muscle during exercise) but not in adductor magnus (inactive-muscle), reflecting the local increase in perfusion with both groups showing a maximum value in the second post-exercise time-point. A significantly greater increase in perfusion from the baseline ($p < 0.05$) was observed in the younger group ($37 \pm 12.05\%$) compared to the older group ($17.57 \pm 15.92\%$) at the first post-exercise measurement. This work establishes a reliable non-invasive method that can be used to study the effects of aging on dynamic changes in muscle perfusion as they relate to important measures of physical function.

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Graphical Abstract

We optimized a diffusion weighted magnetic resonance imaging sequence and validated an intravoxel incoherent motion (IVIM) analysis to study muscle perfusion impairment with aging during post-exercise hyperemia in the thigh muscle. We report IVIM parameter uncertainties based on Monte Carlo simulations and show this approach is sensitive to differences in f_p and D in response to exercise that can be interpreted in the context of the physiology of aging. This method can be used to non-invasively study dynamic changes in muscle perfusion and its consequences on muscle function in aging.

Keywords

Diffusion weighted MRI; IVIM modeling; Monte Carlo simulation; muscle perfusion; dynamic exercise; thigh muscle; aging

Introduction

Aging is associated with impaired endothelium-dependent vasodilation, which contributes to organ dysfunction and leads to the pathogenesis of cardiovascular disease [1, 2]. Impaired muscle perfusion results in inadequate response to changes in oxygen and nutrient demands that occur during and after muscle contraction, potentially causing relative ischemia, muscle damage and functional impairment [3]. Some published studies support this hypothesis and suggest that changes in endothelial-dependent vasodilation with aging are implicated in the pathogenesis of sarcopenia, the decline of muscle strength and mass that is often observed in older persons [1, 4].

Previous clinical studies have attempted to quantify tissue perfusion using arterial injection of microspheres, dynamic contrast-enhanced magnetic resonance imaging [5], single-proton

emission computed tomography, and perfusion Computed Tomography [6, 7]. However, these techniques are invasive and risky to study participants or patients. For example, a microsphere experiment requires arterial injection and subsequent arterial sampling as well as tissue sampling [8]. Therefore, most studies performed using these techniques are small, mostly enroll healthy participants, and therefore have limited generalizability. Diffusion weighted magnetic resonance imaging (DW-MRI), as a non-invasive MR technique, can be used to quantify microvasculature blood volume based on the intravoxel incoherent motion (IVIM) model. This method can also separately characterize the diffusive motion of water molecules within the extra-microvascular tissues, which is quantitatively described by the apparent diffusion coefficient [9, 10]. This approach was originally developed in brain studies [11] and has been successfully used in other organ systems such as liver [12], breast [13], and skeletal muscle [14, 15]. Different studies have used this technique to quantify differences in muscle perfusion at rest and following stimuli in different muscle groups such as forearm [16, 17], shoulder [18, 19], lower back [20], and calf muscle [21]. Diffusion tensor imaging has also been used to characterize fiber micro-architectural properties at rest [22] and following exercise [23]. While the methods described in these studies demonstrate the utility of diffusion MRI for characterizing changes in muscle perfusion and diffusion during hyperemia, their direct application to the study of aging are limited and warrant additional development for direct comparison with key functional aspects of aging. Key functional outcome measures in aging include gait speed and measures of mobility, which are more directly related to large muscle groups in the thigh. Indeed, in the study of aging it is suggested that the thigh muscles may provide more insight into mobility loss than whole body lean tissue mass [24].

The goal of the present work was to optimize a DW imaging acquisition protocol to be used with IVIM analysis in the thigh muscle before and after a dynamic knee extension exercise, with the explicit purpose of establishing a valid technique to characterize age-associated differences in exercise-induced hyperemia. Monte Carlo simulations were performed to quantify the impact of tissue properties, image properties, and acquisition parameters on the IVIM parameter errors. Optimized DW sequence parameters were selected based on the simulation allowing for sufficient accuracy and precision in the IVIM parameter estimates. Reported simulation results include errors based on experimentally measured signal to noise ratio (SNR), an estimated range of residual fat signal, and IVIM tissue parameters observed under both pre-exercise and post-exercise conditions. Finally, we applied this optimized protocol to a small healthy cohort of young and old individuals demonstrating sensitivity to post-exercise hyperemia through observed differences in muscle perfusion and water molecular diffusion.

Materials and methods

Intravoxel Incoherent Motion (IVIM)

The IVIM approach was introduced by Le Bihan in 1988 to separate and quantify the incoherently flowing microvascular blood pool signal from that of the tissue diffusion signal using a multiple b value DW-MRI sequence [11]. The choice of b values influences the sensitivity of the measured signal to microscopic motion of water molecules [9]. For

example, the decay of signal that occurs at low b values contains a contribution from both the microvascular blood pool and thermal molecular diffusion of water. The modeling of microvascular perfusion as an incoherently flowing blood pool presumes a pseudorandom distribution of capillary orientation at the voxel level. Therefore, The IVIM model consists of a bi-exponential decay as a function of b values:

$$S(b) / S_0 = f_p' \exp(-bD^*) + (1 - f_p') \exp(-bD) \quad (1)$$

where S is the observed signal, S_0 is the signal at $b = 0$, f_p' is the relative perfusion fraction of the blood pool with its corresponding pseudodiffusion coefficient, D^* , and D represents the molecular diffusion of water in extra-vasculature tissue. We note that biological factors such as microstructural tissue boundaries and selection of diffusion imaging parameters influence the estimation of these model parameters [10, 25, 26].

In this work, we report the perfusion fraction (f_p) after accounting for blood-tissue relaxation time differences as follows [27]:

$$f_p = f_p' C / [1 - f_p'(1 - C)] \quad (2)$$

where f_p is the corrected perfusion fraction and C is the blood-tissue relaxation correction factor, given by:

$$C = [e^{-\frac{TE}{T_{2,blood}}} \left(1 - e^{-\frac{TR}{T_{1,blood}}} \right)] / [e^{-\frac{TE}{T_{2,tissue}}} \left(1 - e^{-\frac{TR}{T_{1,tissue}}} \right)] \quad (3)$$

Values of T_1 and T_2 for muscle and tissue at 3 Tesla were taken from the literature as follows: $T_{2,blood} = 275$ ms, $T_{1,blood} = 1932$ ms, $T_{2,muscle} = 45$ ms, $T_{1,muscle} = 1412$ ms [28].

Numerical simulation:

Monte Carlo simulations were used to assess the effect of noise and residual unsuppressed fat signal on the bias and dispersion of the IVIM parameters in different conditions. The diffusion-weighted signals were generated using the IVIM model with an additional term for the residual fat signal ($f_f \exp(-bD_f)$), with a fat fraction (f_f) varied from 0 to 18% in increments of 2%, to cover the range of residual fat signal magnitude reported in previous works [29, 30], D_f was fixed to 4×10^{-5} mm²/s, two orders of magnitude smaller than diffusion in free water [29]. Different selections of b values were simulated based on previous experimental studies [20, 21, 31] with these selections intended to provide sufficient sampling of the rapidly diffusing microvasculature blood pool: (1) 0, 10, 20, 50, 100, 200, 400, 800 s/mm² (2) 0, 10, 20, 35, 50, 100, 200, 400, 600, 800 s/mm² (3) 0, 3, 7, 10, 15, 20, 25, 30, 40, 50, 70, 100, 200, 400, 600, 800 s/mm². The input IVIM tissue

parameters (f_p , D^* , and D) were selected based on estimated *in vivo* results. Therefore, simulations were performed with a fixed pseudodiffusion coefficient (D^*) $\sim 20 \times 10^{-2} \text{ mm}^2/\text{s}$; (f_p) $\sim 5\%$, and two different diffusion coefficients (D): $2 \times 10^{-3} \text{ mm}^2/\text{s}$, and $2.4 \times 10^{-3} \text{ mm}^2/\text{s}$.

Additional simulations corresponding to the pre- and post-exercise *in vivo* results (see Table 1) with three different perfusion fractions (f_p): 5%, 10%, and 15%; and two different diffusion coefficients (D): $2 \times 10^{-3} \text{ mm}^2/\text{s}$, and $2.4 \times 10^{-3} \text{ mm}^2/\text{s}$ were performed using the 3rd set of b values which were selected to be used for *in vivo* measurements.

For each simulation, complex-valued signals were simulated with additive zero-mean Gaussian noise to produce the following values of SNR: 20, 30, 40, 50, 70, 100, 150, 200, 300, 400. To estimate the bias and dispersion of the IVIM parameters for each set of simulated conditions, the IVIM model (Equation 1) was used to fit the simulated signals in a single step using a Levenberg–Marquardt algorithm for performing the nonlinear least squares fit. The bias was defined as the difference between the average fit value over 1000 independent noise realizations and the true input value divided by the true input value (i.e. $100 \times (\text{average} - \text{true})/\text{true}$). Dispersion was defined as the standard deviation of the fit parameter over 1000 independent noise realizations divided by the averaged fit value. Both bias and dispersion were reported as percentages.

In vivo measurement:

Eight healthy female volunteers with a habitual level of physical activity during their lifespan were recruited from two different age groups: four younger (21-30 year-old) and four older (60-90 year old) subjects were enrolled. Before any information was collected or procedure performed, participants were given a full description of the study, including any risk associated with it, and informed consent was obtained. Exclusion criteria aside from the standard contraindications to MRI were frailty, severe cardiovascular disease and peripheral artery disease. Individuals were scanned before and after in-magnet exercise in one session.

Experimental design

Participants were positioned supine, feet first, with knees supported at about 120° flexion. Ankles were positioned under pedals of a customized MR-compatible ergometer (Quadspect, Ergospect, Innsbruck, Austria) placed on the bed inside of the magnet. Knees and hips were tightly secured with cushions and straps to ensure comfort and to minimize motion during the protocol without restricting blood flow to muscles. Participants were instructed to use the quadriceps muscles to perform dynamic knee extension exercise by moving the pedal (up and down). After baseline measurements, participants performed exercise using the left leg with a prescribed frequency of 45 repetitions/min, for 2.5 minutes. The resistance of the pedal was fixed at 0.4 bar for all subjects. DW-MRI was acquired prior to exercise and at seven time-points immediately following in-magnet knee extension exercise, occurring every 3.25 minutes for a total of 22.75 minutes. (Figure 1).

MRI acquisition

MR images were acquired using a 3T Achieva MRI scanner (Philips Healthcare, Best, Netherlands) equipped with a two channel SENSE flexible coil positioned anteriorly and posteriorly at the mid-thigh level. A single 22 mm transverse imaging slice was positioned at 60% of the total femur length above the medial epicondyle. Initially, a 2D mDixon sequence with FOV 256×224 mm (voxel size $1 \times 1 \times 22$ mm) was acquired to be used in muscle group selection for region of interest (ROI) analysis. DW images were obtained by using a spin echo single-shot echo-planar imaging (EPI) readout with diffusion sensitization in the slice direction. Imaging parameters included TR=3000 ms, TE=46 ms, SENSE factor 2, FOV 256×225 mm, matrix size 224×224 ; partial Fourier factor 0.6, and 16 b values ($b = 0, 3, 7, 10, 15, 20, 25, 30, 40, 50, 70, 100, 200, 400, 600, 800$ s/mm²; these values were selected based on simulation results). Baseline images acquired prior to exercise were obtained using NSA=20 (acquisition time 16.05 min) for increased SNR under low perfusion conditions. Dynamic post-exercise DW imaging was performed using NSA=4 (acquisition time 3.25 min) to allow for increased temporal resolution during post-exercise hyperemia. Although the reduced NSA in post-exercise measurements results in reduced SNR, the elevated post-exercise perfusion fraction produces more favorable conditions for reliable fitting of IVIM parameters compared with resting conditions, as demonstrated using Monte Carlo simulations. To reduce the deleterious impact of fat signal on our measurements, a triple fat suppression approach was applied combining three different techniques: water-specific excitation, slice-select gradient reversal and spectral attenuated inversion recovery [30]. Signal-to-noise ratio for DW imaging was measured for each subject based on the analysis of the $b=0$ image and a noise image that was obtained using an identical sequence with radiofrequency and gradient pulses turned off (Figure 1).

Post processing

IVIM map analysis;—IVIM maps were produced by fitting Equation 1 to the diffusion signal over all b values in one step. For this fit, a custom written MATLAB (MathWorks, USA) script was implemented on a pixel-wise basis using the Levenberg–Marquardt algorithm for nonlinear least squares fit like what was used in the numerical simulations.

Region of interest analysis;—Regions of interest (ROI) were defined in two different muscle groups: rectus femoris (RF), an active muscle during exercise, and adductor magnus (AM), a non-active muscle (Examples are shown in Figure 5). ROIs were carefully selected to exclude fasciae and fat. The corresponding mDixon images were used as a visual reference to improve the ROI selection in each muscle group. Within each ROI, the IVIM parameters were averaged over all pixels for each subject. Perfusion fraction (f_p), pseudodiffusion (D^*), and diffusion (D) coefficient were then reported as mean (SEM) for different muscle groups by averaging over all participants in each age group.

Signal-to-noise quantification;—The SNR map of the DWI data before and after exercise were calculated to compare with the numerical simulation results. Additionally, the ROIs selected for IVIM analysis e.g. rectus femoris and adductor magnus were copied to the corresponding noise images in the identical location to estimate the relevant SNR for both ROIs in each subject. The SNR was determined from the following expression which uses

the averaged signal from the ROI in the $b = 0$ image in combination with the standard deviation of the signal, measured using the equivalent ROI in the noise scan as described by Yu, et al [32]:

$$SNR = \text{mean}_{\mathbf{r} \in \text{ROI}}(S(\mathbf{r})) / (\sqrt{2 / (4 - \pi)} \cdot \text{sd}_{\mathbf{r} \in \text{ROI}}(N(\mathbf{r}))) \quad (4)$$

where $S(\mathbf{r})$ is the image signal intensity in pixel $\mathbf{r} = (r_x, r_y, r_z)$, $N(\mathbf{r})$ is the corresponding signal intensity in the noise image, and the coefficient $\sqrt{2 / (4 - \pi)}$ accounts for the Rayleigh limit of Rician distribution of noise in magnitude images.

Statistical analysis

Linear mixed-effects (LME) regression analysis with a random intercept was used to test whether the post-exercise IVIM parameters differ significantly from the baseline values (pre-exercise) within and between age groups. In Model 1, time and group were included in the LME model as independent categorical variables. In Model 2, group-by-time interaction terms were added to Model 1 to test if changes over the time course differed by age group. ANOVA was performed to compare the fit of the two models. Furthermore, a paired, two-tailed t-test was performed to compare IVIM parameters between RF and AM muscles. Time-specific two-sample Wilcoxon tests were also performed to compare IVIM parameters between age groups. All analyses were performed using R (Version 3.0.2) software and a p value less than 0.05 was considered statistically significant in all analyses.

Result

Numerical simulation result

The bias and dispersion errors for f_p , D^* and D parameters with different perfusion fractions: $f_p = 5, 10$, and 15% and different diffusivity: $D = 2 \times 10^{-3} \text{ mm}^2/\text{s}$, and $2.4 \times 10^{-3} \text{ mm}^2/\text{s}$ corresponding to the pre- and post-exercise *in-vivo* results calculated with the 3rd set of b values (0, 3, 7, 10, 15, 20, 25, 30, 40, 50, 70, 100, 200, 400, 600, 800 s/mm²) and they have been shown in Figure 2. These simulations cover the broad range of SNR (20 to 400) and fat fraction (0 to 18%) values expected in *in-vivo* DW measurements. Overall the bias in f_p ranged from -8% to 138% and the dispersion in f_p ranged from 1.5% to 70% , respectively. At 0% fat fraction and low perfusion fraction ($f_p = 5\%$), f_p shows positive bias (ranging from 14% to 138%) and large dispersion (ranging from 44% to 70%) at low SNR (< 70) with a small dependence on input value of D . However, both bias and dispersion are greatly reduced at high SNR ($SNR > 200$) regardless of fat fraction and model input parameter values. Indeed, the maximum bias and dispersion of f_p were 14% and 9.5% , respectively, for fat fractions less than 8% and SNR of 200. For higher perfusion fraction ($f_p = 10$ and 15%), as expected during exercise-induced hyperemia, both bias and dispersion of f_p dramatically improve even at lower SNR ($SNR \sim 70$) and fat fraction less than 8% , and they vary from -3.5% to 4.5% and from 1.5% to 20.1% , respectively.

Bias error in D^* is strongly influenced by both fat fraction and SNR, making it a difficult parameter to interpret without a priori measurements of both the underlying fat fraction and

SNR. For example, for SNR = 200 and input parameters of $f_p = 5\%$ and $D = 2 \times 10^{-3} \text{ mm}^2/\text{s}$, simulations show a bias error that monotonically changes with increasing fat fraction from 11.9% to -43.6%. This range is also observed to be strongly related to the underlying input value of f_p and D , thus further complicating the interpretation of D^* . This bias error shows substantial variation over the expected range of experimental fat fractions from 0-8% [30] with errors ranging from 7.1% to -33.12% for an SNR of 200. In contrast to bias, the dispersion of D^* is more dependent on SNR than on fat content, reaching values of 40%, 18%, and 12% for SNR = 200 with f_p values of 5%, 10%, and 15%, respectively.

The bias and dispersion of D ranges from 0.002% to -33% and from 0.5% to 14%, respectively, over all conditions studied. Under conditions comparable to the *in vivo* study, maximum bias and dispersion errors in D are -16.5% and 1.1%, respectively, at fat fractions 8% and SNR = 200. These results indicate the D value as the most reliable IVIM parameter.

The influence of b value selection on bias and dispersion errors is shown in supplementary Figure 1, which reveal negligible differences in error estimates of D for different numbers of b values. In contrast, bias and dispersion are reduced in f_p with greater number of smaller b values. For example, the bias in f_p using the 3rd set of b values (i.e. 16 b values) with $f_p = 5\%$, $D = 2 \times 10^{-3} \text{ mm}^2/\text{s}$, SNR = 200, and fat fraction of 8% is 5.9%. The bias in f_p using the 1st set of b values (i.e. 8 b values) under comparable conditions (i.e. approximately equal-time measurement; SNR ~ 300) is 15.2%. Improvements in estimates of D^* are also observed using a greater number of b values on an equal-time basis. For example, the bias and dispersion errors in D^* using the 1st set of b values with $f_p = 5\%$, $D = 2 \times 10^{-3} \text{ mm}^2/\text{s}$, SNR = 300, and fat fraction = 4% are -22.6% and 34.3%, respectively. In contrast, bias and dispersion errors in D^* using the 3rd set of b values and SNR = 200 are -10.9% and 26.7%, respectively. Based on these comparisons showing lower bias and dispersion errors using the 3rd set of b values, this set of b values was selected for further Monte Carlo simulation and *in vivo* measurements.

In vivo results

All participants successfully performed the in-magnet knee extension exercise. DW images were acquired according to the described study protocol (see Figure 1) in all volunteers. Overall, the relative perfusion fraction increased from the baseline in active muscles following exercise, with a maximum increase at the second post-exercise time points. This elevated f_p slowly returns towards pre-exercise values; this is apparent based on comparison of the example decays in Figure 3, which shows similar slopes for “rest” and “post-exercise 7” but a larger slope at “post-exercise 2”. Figure 4 (top row) shows typical experimental SNR maps for DW images acquired at baseline and post-exercise. The SNR of images obtained at baseline was substantially greater (up to 800) than that of the post-exercise images in all subjects; this is due to a greater number of signal averages in the baseline measurement. The bottom row of Figure 4 shows the same rest and post-exercise SNR maps windowed between values of 0 and 200, demonstrating that SNR values were greater than or equal to 200 for the majority of pixels in the thigh. SNR values for both muscle ROIs were also calculated for each individual. For example, the average SNR at baseline in the RF was

341 in younger adults versus 432 in older adults. Due to reduced signal average, SNR in the RF was slightly lower following exercise with values of 250 in younger adults compared with 326 in older adults. In AM muscle, the average SNR was 321 in younger adults versus 415 in older adults at rest, and 201 and 210 post-exercise, respectively. These reported post-exercise SNR values represent a conservative estimate of the experimental SNR during post-exercise hyperemia, since the peak of hyperemic signal increase occurs at the second time-point and the post-exercise SNR acquisition was obtained after the seventh dynamic DWI acquisition (Figure 1).

We limit the discussion of IVIM results to the perfusion fraction (f_p) and diffusivity (D) parameters due to the large range of bias errors for the parameter D^* shown in Monte Carlo simulations. Representative maps of IVIM parameters f_p and D at rest and following exercise are shown in Figure 5 for a young (27 year-old) and old (90 year-old) adult. Additionally, time-course plots of f_p and D for all subjects based on the ROI analyses are displayed in Supplementary Figure 2. In this figure, parameter trajectories were normalized individually by subtracting each subject's baseline value from the entire time course. Means and standard errors of IVIM parameters are summarized in Tables 1 and 2 for RF and AM muscle, respectively, by averaging over all participants in each age group. Resting IVIM parameters in RF were significantly greater than AM muscle in both age groups ($p < 0.01$). For example, in young adults, f_p values for the RF and AM muscle groups were 4.63 ± 0.62 and 2.09 ± 0.32 , respectively. In older adults f_p values for RF and AM muscle groups were 4.33 ± 0.56 and 1.76 ± 0.11 , respectively. However, no significant difference was found in resting IVIM parameters between the two age groups in each muscle. In all regression analyses, resting values were used as a reference for the quantification of dynamic changes for the respective muscle and age group. In both age groups, after exercise, f_p in RF (active in exercise) increased compared to the baseline and exhibited a peak at the second-time point, reflecting a local increase in perfusion, while there was no substantial change in AM (inactive muscle). These post-exercise time courses in the RF were substantially different between young and old participants as shown in Figure 6. In particular, the percent change of perfusion fraction from the baseline in RF showed a significantly higher ($p < 0.05$) value at the first post-exercise measurement in the younger group ($37 \pm 12.05\%$) compared to the older group ($17.57 \pm 15.92\%$). The f_p increased within the rectus femoris in both groups until approximately 5 minutes after exercise (second post-exercise time-point), where the maximum relative change of perfusion fraction from the baseline was observed to be $f_p = 84.49 \pm 20.45\%$ in young and $f_p = 59.72 \pm 11.64\%$ in old group. Further, a slower return from the maximum perfusion toward the baseline was also observed in the older group (39% decrease over 16.25 min) compared to the younger group (68% decrease over 16.25 min). Similar to the perfusion fraction, diffusivity of tissue water in RF increased significantly following exercise and showed a greater percent change from the baseline in younger adults ($10.64 \pm 1.17\%$) compared to the older adults ($6.15 \pm 1.14\%$) at the first post-exercise time-point. Twenty-one minutes after exercise, diffusivity was still higher than baseline in RF muscle in both age groups. However, younger adults had a faster decline from the maximum value (observed at the second post-exercise time-point) toward the baseline (6% decrease over 16.25 min) compared to older adults (2.06% decrease over 16.25 min). Analysis using the linear mixed effects model with the group-by-time interaction terms (Supplemental Table

1) demonstrated a significantly different time course for diffusivity in the two age groups ($p < 0.05$). Unlike diffusivity, perfusion fraction did not show a significant age-dependent dynamic change in post-exercise measurements.

Discussion

This study proposes a DW acquisition scheme that has been optimized for quantifying age-related differences in IVIM-derived muscle perfusion both before and after exercise. The bias and dispersion of IVIM-derived parameters were determined for a range of expected tissue properties and acquisition parameters using Monte Carlo simulations. Based on the simulation results, a large number of small b values (0, 3, 7, 10, 15, 20, 25, 30, 40, 50, 70, 100, 200, 400, 600, 800 s/mm²) with an adequate number of averages in DW sequence (NSA = 20 for rest, and NSA = 4 for post-exercise hyperemia) and a triple fat suppression technique were selected for *in vivo* measurements to reduce bias and dispersion of the IVIM-parameters. Simulation results show these that these acquisition parameters, under our *in vivo* experimental conditions, produce bias errors in f_p and D to within 14% and from -16.5%, respectively. The optimized protocol was demonstrated in a small healthy cohort of young and old individuals to examine its sensitivity to age-associated differences in post-exercise hyperemia. The *in vivo* experimental results showed that the post-exercise change of f_p in RF (active muscle during exercise) from baseline was significantly greater at the first post-exercise measurement in the younger group compared to the older group. Additionally, f_p in the young group showed a more rapid return from the maximum toward the baseline value. Similarly, diffusivity increased following exercise and showed a greater increase from the baseline in younger adults compared to the older adults at the first post-exercise time-point.

Precision and stability of the IVIM parameters

The Monte Carlo simulations demonstrate more reliable IVIM parameter estimates using a larger number of b values. However, even when 16 b values are used, estimation of D^* is still challenging and it has the poorest reliability among all the IVIM parameters. These results are consistent with previous work [33] corroborating that D^* estimation is challenging at clinically achievable SNRs and fat suppression efficiency. In contrast, f_p and D showed a smaller variation in dispersion, from 1.6% to 70% and from 0.5% to 14%, respectively, across all the simulated conditions. These results demonstrate that the accuracy of IVIM parameters strongly depended on fat fraction and SNR. However, owing to the optimization of our DW sequence by applying a triple fat suppression technique in this work, the amount of residual fat signal is not expected to exceed 8% [30]. Furthermore, the level of SNR across all the *in-vivo* measurements was optimized to be approximately 200 or more. As a result of these optimizations, the maximum error of dispersion in our *in-vivo* results was substantially reduced in all IVIM parameters as follows: 40% for D^* , 16% for f_p , and 2% for D according to the Monte Carlo simulation. Additionally, these simulations demonstrate that stability and reliability of IVIM analysis in active muscle should be better in post-exercise measurements than at rest due to the increase of perfusion fraction with exercise. Overall, a high SNR (> 200), as observed in our measurements, and sufficient number of smaller b

values in the range of 0 to 50 s/mm² as well as appropriate fat suppression improved the IVIM model parameter estimates, particularly with regards to the estimation of f_p .

Comparison of the IVIM values with previously reported data

The IVIM-derived parameters measured at baseline in different muscle groups showed meaningful differences. This distinction can be interpreted according to the different diffusion sensitization of measurement with fiber orientation, and different fiber type in each muscle group as it was previously reported by Mastropietro et al. [21]. For example, RF is largely composed of fast twitch (type II) muscle fibers arranged in a bipenniform manner with the deep fibers oriented straight down to the deep aponeurosis [34]. In contrast, the AM muscle has more slow twitch fibers (type I) and is located on the inner thigh, originating from the ischium and descending to the femur [35, 36]. Therefore, RF muscle fibers are mainly oriented along the diffusion sensitization direction of the DW sequence (slice direction) while the AM fibers are largely oblique to this direction. Consequently, smaller perfusion and diffusivity are expected in AM muscle compared to RF. In general, the f_p values measured at rest for different age groups in this study are close to those found by Morvan ($3 \pm 1\%$) and Filli et al. ($4 \pm 3\%$) in forearm [16, 17], and Mastropietro et al. ($6.8 \pm 1.3\%$) in gastrocnemius [21]. Values of D in both ROIs are slightly higher compared with observations by Nguyen [19] in shoulder ($1.52 \pm 0.04 \cdot 10^{-3} \text{ mm}^2/\text{s}$) and by Filli [17] in forearm ($1.43 \pm 0.06 \cdot 10^{-3} \text{ mm}^2/\text{s}$); this can be explained by the size and properties of each muscle as well as different diffusion directions of measurements. For example, Filli and Nguyen used a three-orthogonal direction sensitization, while we applied sensitization only in the slice direction and thus our slightly elevated diffusion values could be due to our measurement being sensitized along the muscle fiber where there are fewer restrictions, as described above. Additionally, the blood flow pseudodiffusion coefficient measured in this work in both ages (Example from young group $D^* = 21.5 \pm 1.5 \cdot 10^{-2} \text{ mm}^2/\text{s}$ in RF at rest) is virtually one order of magnitude greater than the previous published reports (for example $D^* = 28.5 \pm 11.4 \cdot 10^{-3} \text{ mm}^2/\text{s}$) in forearm [17], shoulder [18], and gastrocnemius [23]; this difference can be explained by differences in the range of b values. In this work, we acquired 10 b value weightings between 0 and 50 s/mm² whereas others use substantially fewer (e.g. 4 b values between 0 and 50 s/mm² are used in [21]), making our measurements more sensitive to perfusion as demonstrated by Monte Carlo simulations. Another factor that can be contributed to a lower estimate for the D^* is the influence of residual fat signal from less efficient fat suppression, which is particularly apparent when fewer b values are measured. This shows the importance of fat suppression for D^* measurement particularly in the subjects that have higher fat infiltration. Furthermore, the estimation of IVIM-derived parameters, particularly pseudodiffusion coefficient, is strongly influenced by SNR. The SNR of our in vivo images were optimized to have an average SNR 200, which is greater than that reported in previous work [21].

Dynamic changes following exercise and age differences

Dynamic changes in IVIM values were observed in RF muscle following exercise in both age groups. Among all the IVIM parameters, perfusion fraction showed the largest percent change from the baseline during the post-exercise hyperemia in both young and old groups that occurs at the second time-point. Additionally, a smaller f_p in the second post-exercise

time-point was observed in older participants ($6.72\% \pm 0.60$) compared with that in younger participants ($8.07 \pm 0.38\%$). Overall, the significant increase of f_p in RF at the first post-exercise measurement in younger adults compared to the older adults demonstrates a similar trend to a previous aging study by Schulte et al. [37]. The authors reported a shorter time to peak muscle perfusion in younger participants (30.3 ± 6.5 years) compared to the older (64.0 ± 6.4 years) for post-occlusive reactive hyperemia measured using a BOLD MRI technique. In another aging study using arterial spin labeling (ASL) MRI [38], authors showed a reduced perfusion signal in gastrocnemius/soleus muscle at the end of 5 minutes planter flexion exercise in the elderly adults (70 ± 2 years) compared to the younger adults (26 ± 2 years). Although, the basal perfusion is not report in either of these studies [37, 38] the age-associated perfusion differences in post-exercise hyperemia are consistent with our IVIM findings. The physiological basis for this observation might be the decline of active capillaries with aging, an increase in vessel wall rigidity, or endothelium impairment [1, 33]. In addition, we observed a slower return of f_p towards the baseline value in older adults. These observations may be explained by the impaired skeletal muscle blood flow regulation during exercise with aging [3]. The etiology of this impairment is multifactorial; lower density of active capillaries, deterioration of endothelium-dependent vasodilation, as well as dysfunction of the sympathetic nerve system are important contributors [1, 3].

One of the key findings in this study is the significant age-associated difference in water molecular diffusion from the extra-vasculature space (D). This result suggests that in addition to the muscle perfusion, the diffusivity of muscle during post-exercise hyperemia is a potentially interesting metric of skeletal muscle in aging. Indeed, this is a strong advantage of the present work in comparison to previous perfusion studies, namely those using ASL and BOLD techniques [37, 38]. Our in vivo results show that similar to f_p , diffusivity increased in the active muscles (RF) as a result of exercise in both age groups. This finding corresponds with previous studies of post-exercise hyperemia [17, 19]. Interestingly, the post-exercise changes observed in diffusivity follow the same pattern as observed for f_p , demonstrating the contribution of blood flow changes to diffusivity. Luciani and colleagues initially suggested that the observed increase in apparent diffusion coefficient might be influenced by both tissue water diffusion and perfusion by microvasculature [12]. Filli and colleagues argued that although increases in temperature after exercise can theoretically increase diffusivity, this is unlikely to be the primary cause. They pointed out a lack of diffusion increase in adjacent muscles and the observed increase in muscle signal at $b=0$ after exercise, indicating cellular and interstitial edema and supporting the influence of perfusion. In summary, a definitive explanation is still under debate and further measurements are needed to address this question. Finally, diffusivity remained significantly elevated during the entire post-exercise measurement time of 22 minutes ($p < 0.05$). Values of f_p were also observed to be slightly higher than the baseline after 22 minutes recovery following the exercise. These findings are in agreement with previous studies in forearm [17] and masticatory muscle [39] and can be explained by oxygen deficit or a low pH as a result of metabolic substrate accumulation. However, this is in contrast with a recent study in calf muscle [21], which utilized a different type of exercise (isometric exercise with 50% of the actual maximum voluntary plantar-flexion torque as the target force).

In contrast to active muscle, the IVIM values show a decrease in diffusion within the inactive muscle (AM) during post-exercise hyperemia. We note that the measurement variance of D is large relative to the observed post-exercise change in the inactive muscle and so this result should be confirmed using a larger sample size. This observation is consistent with the known response of the vascular system to redirect blood flow away from muscle with less metabolic demand (e.g. the AM muscle) and toward muscle with greater metabolic demand (quadriceps muscles in this exercise).

Limitations of this work

Overall, the IVIM approach has been optimized for quantification of perfusion and diffusivity in skeletal muscle with sufficient accuracy and precision. It has been shown that the IVIM parameter bias and dispersion errors due to the influence of factors such as low SNR and residual fat signal can be characterized and minimized based on Monte Carlo simulation. In addition to the above analysis that highlights the predominant sources of error using IVIM in skeletal muscle, some additional limitations should also be considered. The single-shot EPI sequence used in combination with a two-channel flex coil and a SENSE factor of 2 resulted in a SENSE image artifact in the vastus lateralis muscle, restricting our ROI analysis to rectus femoris for highly active muscle groups during exercise. A smaller SENSE factor can be used to decrease the SENSE artifact; however, this results in a substantially increased fat shift artifact. Even with triple fat suppression which suppresses the majority of the fat signal [30], large spatial shifts of imperfectly suppressed subcutaneous fat can potentially impact IVIM parameter estimates. In this work, we sought to balance this tradeoff between SENSE factor and fat shift artifact. The age group differences in post-exercise hyperemia reported in this work are consistent with what others have reported using alternative techniques. These preliminary results should be confirmed in a larger population.

Conclusion

We optimized a DW MRI sequence to accurately quantify muscle perfusion in the quadriceps based on the IVIM model. The reliability of the in vivo results was supported by Monte Carlo simulation and it has been demonstrated that under achievable clinical SNR (SNR ~ 200) and minimum fat contamination (less than 8%) with adequate number of small b values (10 values ranging from 0 to 50 s/mm²) the bias and dispersion of f_p ranged from -3.3% to 14% and from 3.2% to 9.5%, respectively. This approach, when applied to a young and old healthy cohort, clearly shows a different trend in post-exercise hyperemia in younger and older age groups that can be interpreted in the context of age-associated changes in microvascular function as discussed in this work. In clinical research, the application of this non-invasive method to measure muscle perfusion can aid in the development of strategies to treat or prevent age-related disease such as sarcopenia where microvascular impairment is involved.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

DW	diffusion-weighted
IVIM	intravoxel incoherent motion
ASL	arterial spin labeling
SNR	signal to noise ratio
f_p	perfusion fraction
D^*	pseudodiffusion coefficient
D	diffusion coefficient
EPI	echo planar imaging
TR	repetition time
TE	echo time
ROI	region of interest
RF	rectus femoris
AM	adductor magnus
LME	Linear mixed-effects
SEM	standard error of the mean

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Figure 1.

Examination protocol for the MRI acquisition including diffusion weighted imaging (DWI) acquisition at rest (16.05 min), and post exercise (22.75 min). The noise images were also acquired at rest and in following of the last post-exercise measurement to calculate the signal-to-noise ratio (SNR). The exercise was performed inside the magnet with a MR compatible ergometer.

Figure 2.

Plots of bias and dispersion (%) for all IVIM parameters in Monte-Carlo simulations. Results are shown for two different input values of diffusion coefficient (D), as obtained from the rectus femoris (RF) muscle in the pre- and post-exercise measurements: $D = 2 \times 10^{-3} \text{ mm}^2/\text{s}$ and $D = 2.4 \times 10^{-3} \text{ mm}^2/\text{s}$. Columns denote different values for f_p measured in the RF before and after exercise. D^* was fixed at $20.0 \times 10^{-2} \text{ mm}^2/\text{s}$, and an additional term for the residual fat signal ($f_f \cdot e^{(-D_f t^b)}$) was added to the IVIM model. D_f was fixed at $4 \times 10^{-5} \text{ mm}^2/\text{s}$, and fat fraction (f_f) varied from 0 to 18% in steps of 2% as shown on the x-axis. The signal-to-noise ratio (SNR) were incremented from 20 to 400 in different steps as follows: 20, 30, 40, 50, 70, 100, 150, 200, 300, 400.

Figure 3. Example of signal decay averaged over all pixels in the RF muscle for a 30 year-old adult at rest and post-exercise. The inset shows a zoomed view for b values from 0 to 100 s/mm² with a log scale for the y axis.

Figure 4.

Examples of SNR maps at rest and post-exercise for a 21 year-old volunteer (top row). The bottom row shows the same SNR maps scaled from 0 to 200. These SNR data can be matched with the Monte Carlo simulation results shown in Figure 2 to estimate the bias and dispersion of the IVIM parameters from our in vivo results.

Figure 5.

Representative perfusion fraction (f_p) and diffusion (D) maps at rest and following exercise from a series of post-exercise measurements (see Fig. 1) on young (27 year-old) and old (90 year-old) adults. Regions of interest (ROIs) were drawn on the baseline images to include the rectus femoris (RF), shown in red, and a part of the adductor magnus (AM), shown in white. These were used to generate the quantitative data shown in the following figures and tables.

Figure 6.

Quantitative results for perfusion fraction (f_p) and diffusivity (D) in the rectus femoris (left column) and adductor magnus (right column) muscles at rest and following exercise. Data points represent means $\pm$ SEM. ($\dagger$) above or below a data point refers to a significant change compared to rest.

Table 1.

Quantitative values of perfusion fraction (f_p), pseudo-diffusion coefficient (D^*), and diffusion coefficient (D) in rectus femoris at rest and following exercise calculated from the region of interest analysis. Data are represented as means $\pm$ standard error, after averaging over all participants in the same age group as young (left column) and old (right column). ($\dagger$) above a data point refers to a significant change compared to rest.

Active muscle	Young			Old		
	f_p [%]	D^* [10^{-2} mm 2 .s $^{-1}$]	D [10^{-3} mm 2 .s $^{-1}$]	f_p [%]	D^* [10^{-2} mm 2 .s $^{-1}$]	D [10^{-3} mm 2 .s $^{-1}$]
Rest	4.63 $\pm$ 0.62	21.42 $\pm$ 1.55	2.19 $\pm$ 0.03	4.33 $\pm$ 0.56	20.47 $\pm$ 1.35	2.22 $\pm$ 0.04
Exercise						
Post-Exe 1	6.26 $\pm$ 0.89 $\dagger$	17.54 $\pm$ 1.54	2.43 $\pm$ 0.03 $\dagger$	5.10 $\pm$ 0.98	24.19 $\pm$ 5.52	2.35 $\pm$ 0.03 $\dagger$
Post-Exe 2	8.07 $\pm$ 0.38 $\dagger$	16.95 $\pm$ 2.27	2.48 $\pm$ 0.03 $\dagger$	6.72 $\pm$ 0.60 $\dagger$	21.19 $\pm$ 1.83	2.44 $\pm$ 0.01 $\dagger$
Post-Exe 3	6.75 $\pm$ 0.32 $\dagger$	22.45 $\pm$ 2.03	2.43 $\pm$ 0.03 $\dagger$	6.27 $\pm$ 0.50 $\dagger$	22.51 $\pm$ 1.00	2.39 $\pm$ 0.03 $\dagger$
Post-Exe 4	6.20 $\pm$ 0.34 $\dagger$	24.23 $\pm$ 1.59	2.39 $\pm$ 0.03 $\dagger$	5.90 $\pm$ 0.51 $\dagger$	23.30 $\pm$ 2.28	2.37 $\pm$ 0.02 $\dagger$
Post-Exe 5	5.69 $\pm$ 0.36 $\dagger$	25.73 $\pm$ 0.73	2.35 $\pm$ 0.04 $\dagger$	5.51 $\pm$ 0.43 $\dagger$	23.97 $\pm$ 1.81	2.34 $\pm$ 0.02 $\dagger$
Post-Exe 6	5.48 $\pm$ 0.46	25.14 $\pm$ 1.09	2.32 $\pm$ 0.04 $\dagger$	5.22 $\pm$ 0.45	24.04 $\pm$ 2.20	2.32 $\pm$ 0.02 $\dagger$
Post-Exe 7	5.25 $\pm$ 0.48	22.13 $\pm$ 1.2	2.30 $\pm$ 0.03 $\dagger$	5.05 $\pm$ 0.46	22.95 $\pm$ 1.92	2.30 $\pm$ 0.02 $\dagger$

Table 2.

Quantitative values of perfusion fraction (f_p), pseudo-diffusion coefficient (D^*), and diffusion coefficient (D) in adductor magnus at rest and following exercise calculated from the region of interest analysis. Data are represented as means $\pm$ standard error, after averaging over all participants in the same age group as young (left column) and old (right column). ($\dagger$) above a data point refers to a significant change compared to rest.

Non-active muscle	Young			Old		
	f_p [%]	D^* [10^{-2} mm 2 .s $^{-1}$]	D [10^{-3} mm 2 .s $^{-1}$]	f_p [%]	D^* [10^{-2} mm 2 .s $^{-1}$]	D [10^{-3} mm 2 .s $^{-1}$]
Rest	2.09 $\pm$ 0.32	11.90 $\pm$ 1.80	1.81 $\pm$ 0.01	1.76 $\pm$ 0.11	14.91 $\pm$ 1.71	1.87 $\pm$ 0.03
Exercise						
Post-Exe 1	1.44 $\pm$ 0.17	11.04 $\pm$ 2.44	1.76 $\pm$ 0.03 $\dagger$	1.48 $\pm$ 0.27	12.48 $\pm$ 2.29	1.83 $\pm$ 0.02
Post-Exe 2	2.21 $\pm$ 0.09	15.02 $\pm$ 1.76	1.79 $\pm$ 0.03	1.63 $\pm$ 0.11	11.65 $\pm$ 2.10	1.82 $\pm$ 0.01 $\dagger$
Post-Exe 3	2.22 $\pm$ 0.24	17.37 $\pm$ 1.83 $\dagger$	1.81 $\pm$ 0.02	1.33 $\pm$ 0.18	11.75 $\pm$ 2.16	1.82 $\pm$ 0.02 $\dagger$
Post-Exe 4	2.58 $\pm$ 0.18	16.34 $\pm$ 2.65 $\dagger$	1.79 $\pm$ 0.02	1.63 $\pm$ 0.43	12.48 $\pm$ 1.72	1.83 $\pm$ 0.02
Post-Exe 5	2.15 $\pm$ 0.30	17.35 $\pm$ 2.21 $\dagger$	1.81 $\pm$ 0.01	1.68 $\pm$ 0.25	12.45 $\pm$ 0.85	1.83 $\pm$ 0.02 $\dagger$
Post-Exe 6	2.47 $\pm$ 0.23	15.19 $\pm$ 2.49	1.81 $\pm$ 0.01	1.91 $\pm$ 0.53	12.82 $\pm$ 1.54	1.82 $\pm$ 0.01 $\dagger$
Post-Exe 7	2.10 $\pm$ 0.28	14.87 $\pm$ 2.57	1.81 $\pm$ 0.01	1.68 $\pm$ 0.24	12.35 $\pm$ 1.90	1.83 $\pm$ 0.01 $\dagger$