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Repeatability and Variability of Myocardial Perfusion Imaging Techniques in Mice: Comparison of Arterial Spin Labeling and First-pass Contrast-enhanced MRI

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Abstract

Purpose—Preclinical imaging of myocardial blood flow (MBF) can elucidate molecular mechanisms underlying cardiovascular disease. We compared the repeatability and variability of two methods, first-pass MRI and arterial spin labeling (ASL), for imaging MBF in mice.

Methods—Quantitative perfusion MRI in mice was performed using both methods at rest, with a vasodilator, and one day after myocardial infarction (MI). Image quality (score of 1–5, 5 best), between-session coefficient of variability (CV_{bs}), intra-user coefficient of variability ($CV_{intra-user}$) and inter-user coefficient of variability ($CV_{inter-user}$) were assessed. Acquisition time was 1–2 minutes for first-pass MRI and approximately 40 minutes for ASL.

Results—Image quality was higher for ASL (3.94 ± 0.09 vs. 2.88 ± 0.10 , $p < 0.05$). Infarct zone CV_{bs} was lower with first-pass ($17 \pm 3\%$ vs. $46 \pm 9\%$, $p < 0.05$). The stress perfusion $CV_{intra-user}$ was lower for ASL ($3 \pm 1\%$ vs. $14 \pm 3\%$, $p < 0.05$). The stress perfusion $CV_{inter-user}$ was lower for ASL ($4 \pm 1\%$ vs. $17 \pm 4\%$, $p < 0.05$).

Conclusion—For low MBF conditions such as infarct, first-pass MRI is preferred due to better repeatability and variability. At high MBF such as at vasodilation, ASL may be more suitable due to superior image quality and lower user variability. First-pass MRI has a substantial speed advantage.

Keywords

myocardial perfusion imaging; cardiac MRI; mouse; ASL; first-pass MRI

Introduction

The assessment of myocardial blood flow (MBF), or perfusion, is central to the evaluation of ischemic heart disease. MBF is markedly reduced in myocardial infarction (MI), and the

recovery of MBF to injured tissue is critical to infarct healing and to emerging strategies for cardiac regeneration. Abnormal myocardial perfusion reserve (MPR), the ratio of MBF at stress to rest, is widely used to evaluate ischemia and detect obstructive coronary artery disease (CAD). MPR can also be reduced in the absence of obstructive CAD (1–3), such as in diabetes (2,4), obesity (5), metabolic syndrome (6), and other conditions where microvascular dysfunction limits the ability to augment MBF. Reduced MPR is prognostic of adverse cardiac events in patients with and without obstructive CAD (4), and in many diseases involving microvascular dysfunction. The molecular and cellular mechanisms underlying reduced MPR in the absence of obstructive CAD are not well understood.

Preclinical research across the full spectrum of heart diseases, including infarct healing, cardiac regeneration, and microvascular disease, is largely performed in small animals such as mice and rats. Recent preclinical studies where quantitative MRI of MBF provided critical data include a study by Zhang *et al.* (7) where MRI quantified temporal changes in MBF after transplantation of endothelial cells to the infarcted heart, a study by Banquet *et al.* (8) where MRI perfusion imaging demonstrated the therapeutic benefit of a growth factor combination therapy in a chronic heart failure mouse model, and a study by Hiller *et al.* (9) where MRI showed an improvement in microvascular function after treatment with a tissue-specific angiotensin converting enzyme (ACE) inhibitor after coronary stenosis.

Two different MRI methods, first-pass contrast-enhanced MRI and arterial spin labeling (ASL), can be used to quantify MBF in small animals. Although first-pass imaging is the MRI method of choice to assess myocardial perfusion in humans, due to technical constraints related to spatiotemporal resolution, the historically superior option for imaging myocardial perfusion in small animals was ASL (10–13). More recently, with the advancement of acceleration methods, first-pass perfusion imaging has become technically feasible in mice and rats, and first-pass methods that quantify MBF in small animals have been reported (14–18). Since two methods, ASL and first-pass MRI, are now available, we sought to evaluate them for imaging MBF in mice over a wide range of blood flows, and to compare their advantages and disadvantages under a variety of conditions. Specifically, we compared repeatability, variability, and image quality of ASL versus first-pass MRI in mice under a range of conditions.

Methods

Experimental Design

Wild-type male C57Bl/6 mice (n=7) were imaged at rest, with vasodilation using Regadenoson (Lexiscan, Astellas Pharmis, 0.1µg/g body weight) and after MI (separate group, n=7), representing conditions of intermediate, high, and low MBF, respectively. Under each condition, mice were imaged using both a first-pass sequence (16) and a flow-sensitive alternating inversion-recovery (FAIR) Look-Locker ASL sequence. To assess repeatability, perfusion imaging for each technique was performed at two different sessions. The repeated rest and stress imaging sessions were separated by approximately one week in order to allow the mice sufficient time to recover between sessions. The infarcted mice underwent the first session on day 1 post-MI and second session on day 2 post-MI in order to allow sufficient time for gadolinium to wash out from the infarcted tissue and to minimize

changes in perfusion that may occur during infarct healing (13). For the rest and stress groups, no specific order was used in performing the techniques (ASL vs. first-pass) and furthermore, the ASL and first-pass sessions were separated by approximately one week to allow the mice sufficient time to recover from isoflurane and Regadenoson. However for both techniques, rest perfusion imaging was performed before stress perfusion imaging because the half-life of Regadenoson is approximately 60 minutes (19), which precludes the option of performing stress prior to rest imaging during a single exam. In all the post-MI mice, ASL was performed first, followed by first-pass MRI because ASL is best performed in the absence of gadolinium-DTPA. Perfusion estimates obtained at rest, stress and post-MI using both techniques were compared for between-session repeatability, intra-user and inter-user variability and inter-animal variability. Overall image quality of the perfusion images was also assessed by two readers using a 5-point scale (1-very poor, 2-poor, 3-good, 4-very good, 5-excellent), where judgment of high quality was largely influenced by low levels of aliasing and motion artifacts.

Animal Handling

All animal studies were performed under protocols that comply with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85–23, Revised 1996) and were approved by the Animal Care and Use Committee at our institution. An indwelling tail vein catheter was established to deliver gadolinium-DTPA (Magnevist, 0.1mM/kg body weight) and Regadenoson (Lexiscan, Astellas Pharmis, 0.1µg/g body weight). Mice were positioned supine in the scanner and body temperature was maintained at $36\pm0.5^{\circ}\text{C}$ using thermostated circulating water. Anesthesia was maintained using 1.25% isoflurane in O_2 inhaled through a nose cone during imaging. The ECG, body temperature and respiration were monitored during imaging using an MR-compatible system (SA Instruments, Stony Brook, New York). MI was induced by permanent ligation of the left anterior descending coronary artery (20).

MRI Hardware

MRI was performed on a 7T Clinscan system (Bruker, Ettlingen, Germany) equipped with actively shielded gradients with a full strength of 650mT/m and a slew rate of 6666 mT/m/ms. A 30mm diameter birdcage RF coil with an active length of 70 mm was used.

MRI Pulse sequences and Imaging Protocol

ASL—ASL was performed using a compressed-sensing (CS) accelerated spiral FAIR Look-Locker sequence, which is based on a previously developed cardio-respiratory gated (CRG) FAIR ASL sequence that has been used to measure perfusion in the mouse heart at rest, with vasodilation and post-MI (13). Using this sequence, upon detection of a CRG trigger, a non-selective or slice-selective inversion was applied. For 50–60 triggers after the inversion, an RF pulse was applied and a spiral gradient echo was acquired. The spiral interleaves were undersampled at rate-2 acceleration. Other imaging parameters included: time between inversions = 7s, slice thickness = 1 mm, thickness for slice-selective inversion = 2.5 mm, number of spiral interleaves for full Nyquist sampling = 87, pixel size = $100 \times 100 \mu\text{m}^2$, and averages = 3. A hyperbolic secant RF pulse was used for inversion, where the inversion efficiency as measured in a saline phantom was 99%. A 3° excitation pulse flip angle was

used to minimize perturbation of recovering longitudinal magnetization. Look-Locker image sets were acquired after slice-selective and non-selective inversions. The total acquisition time for one session of CS-accelerated ASL was approximately 40 minutes.

In all mice, localizer imaging was performed to select a mid-ventricular short-axis slice. Rest ASL imaging was performed first, followed by stress ASL. For stress ASL imaging, Regadenoson was injected i.v. and about 7–10 minutes later the slice-selective acquisition was performed, followed by the non-selective acquisition.

First-Pass MRI—First-pass MRI was performed using a previously described CS-accelerated dual-contrast saturation-recovery gradient echo sequence (16). Briefly, two short-axis slices (slice gap = 0.2mm) were acquired within each cardiac cycle, one to obtain the arterial input function (AIF) and the other to obtain the tissue function (TF). Pulse sequence parameters included: FOV = $25.6 \times 18\text{mm}^2$, phase resolution = 80%, matrix = 128×74 , pixel size = $200 \times 250\mu\text{m}^2$, TE/TR = 1.2/2.1ms, flip angle = 15° , slice thickness = 1mm, AIF saturation delay = 15ms, TF saturation delay = 57ms, acceleration rate = 6 for the AIF and 4 for the TF, AIF acquisition time = 25ms/image and TF acquisition time = 36ms/image. Twelve phase-encode lines were acquired for each AIF image and 17 lines were acquired for each TF image.

In all mice, localizer imaging was performed to select a mid-ventricular short-axis slice. A series of 250 first-pass images were acquired using the dual-contrast sequence. Ten seconds after the acquisition started, the contrast agent was injected i.v. while imaging continued to capture the first-pass kinetics. For the stress-perfusion acquisition, Regadenoson was injected i.v. and first-pass imaging was performed 10 minutes later.

Compressed Sensing Reconstruction—First-pass MRI and ASL both utilized data undersampling for acceleration, and both were reconstructed using a recently-developed CS algorithm called Block Low-rank Sparsity with Motion-guidance (BLOSM) (21). This method applies low rank sparsity to regions of images, and can also use inter-frame region tracking for motion compensation. BLOSM with motion compensation was used to reconstruct the undersampled free-breathing first-pass images, and BLOSM without motion compensation was used to reconstruct the respiratory-gated undersampled ASL images. The advantages of regional low rank sparsity for perfusion imaging have been described previously (21).

Perfusion Analysis—Tracer-kinetic modeling was used to quantify MBF from both first-pass and ASL images. For first-pass perfusion analysis, the signal intensity – time curve for the AIF was generated by placing a region of interest (ROI) in the left ventricular (LV) blood pool in the images acquired using a short saturation delay. Similarly, the signal intensity – time curve for the TF was generated by placing a ROI in the myocardial tissue in the images acquired using the longer saturation delay. One TF ROI representing all of the myocardium observed in the slice was used. The signal intensities of the T1-weighted first-pass images were normalized by the signal intensities of proton density images (acquired at the end of the first-pass acquisition) and then they were converted into T1 values using methods described by Cernicanu and Axel (22). The relaxivity of gadolinium was assumed to be

3.8L/mmol.s (23), which was used to convert the T1 values to gadolinium concentrations. The AIF curve was fit using a gamma-variate function in order to denoise the data and remove effects due to the recirculation of gadolinium. Using data representing first-pass kinetics (but not recirculating gadolinium), the fitted AIF and TF were analyzed using Fermi function deconvolution (24) to estimate myocardial perfusion (16).

For ASL analysis, ROIs comprising the myocardial tissue of the Look-Locker images acquired after slice-selective (SS) and non-selective (NS) inversions were used to generate signal intensity-time curves ($S_{SS}(t)$ and $S_{NS}(t)$). The blood signal intensity-time curve ($S_b(t)$) was measured by placing a ROI in the LV blood pool of the NS images. $T1_{NS}$, $T1_{SS}$ and $T1_{blood}$ were estimated by fitting $S_{NS}(t)$, $S_{SS}(t)$ and $S_b(t)$ to a two-parameter monoexponential curve (Eq. 1) using a non-linear least squares fitting algorithm.

$$S(t)=S_0 \left(1 - 2 \times \exp \left(\frac{-t}{T_1} \right) \right) \quad (\text{Eq. 1})$$

In this equation, $S(t)$ denotes the signal intensity and S_0 is the proton density. The proton densities for the myocardium and blood were also determined from the two-parameter fits. The signal intensities for the myocardium ($S_{NS}(t)$, $S_{SS}(t)$) and blood ($S_b(t)$) were normalized by the respective proton densities (S_0) to generate longitudinal magnetization-time curves for myocardium ($M_{NS}(t)$ and $M_{SS}(t)$) and blood ($M_b(t)$). Cubic splines were then used to interpolate $M_{NS}(t)$ and $M_{SS}(t)$ in time, and the difference curve, $D(t)$, was generated using the difference between the spline fits of $M_{NS}(t)$ and $M_{SS}(t)$. $D(t)$ was then fit to an ASL kinetic model in order to estimate perfusion. The ASL kinetic model is described in detail in Appendix 1. If we define T_{1app} as the apparent T_1 of the myocardium given by

$\frac{1}{T_{1app}} = \frac{1}{T_1} + \frac{f}{\lambda}$, then, using the kinetic model, the difference function $D(t)$ can be written as:

$$D(t)=2fS_B^0W(t)e^{-t/T1B} * e^{-t/T1app} \text{ where } W(t)=\int_{\tau=0}^t h(\tau)d\tau \text{ and } h(t)=(t-t_{delay})^\alpha e^{-(t-t_{delay})/\beta}$$

where f is flow in s^{-1} , S_B^0 is the proton density of blood, $\lambda=0.95$ is the blood/tissue water partition coefficient (11), $T1B$ is the relaxation time constant of blood, $W(t)$ is the input function, and $h(t)$ is the blood transit time distribution (dispersion function) that takes the form of a gamma-variate. For $h(t)$, α determines the upslope, β determines the decay of $h(t)$ and t_{delay} is the transit delay. All image analysis was performed using MATLAB (The Mathworks, Natick, MA, USA).

Repeatability and data variability—Bland-Altman analysis was used to compare the between-session repeatability and the intra- and inter-user variability of the two techniques. The Bland-Altman repeatability coefficient (RC), which represents the 95% confidence interval of the differences in perfusion estimates between sessions, was calculated and normalized to the mean perfusion estimate and expressed as a percentage. The coefficient of variability (CV) is defined as the standard deviation of the datasets normalized by the mean value and expressed as a percentage. The between-session coefficient of variability (CV_{bs})

was calculated for each animal by comparing the perfusion estimates between session 1 and session 2 and the mean CV_{bs} and standard error of mean (SEM) were expressed for both techniques.

The perfusion estimates from both sessions were analyzed by the same user twice and the mean and SEM for the intra-user coefficient of variability ($CV_{intra-user}$) were calculated by comparing the perfusion estimates between the two analysis sessions. Similarly, all the datasets were analyzed by two different users and the inter-user coefficient of variability ($CV_{inter-user}$) was calculated by comparing the perfusion estimates between the two users.

The inter-animal coefficient of variability (CV_a) for each session was calculated by normalizing the standard deviation of perfusion values from different animals to the mean perfusion value of that session. Using CV_a from both sessions, a mean and SEM were estimated for both first-pass MRI and ASL.

Statistical Analysis—All values in the text, tables and figures are expressed as mean $\pm$ SEM. The CV_{bs} , $CV_{intra-user}$, and $CV_{inter-user}$ values were compared between the two techniques using a paired t-test. Image quality scores were compared using the Mann-Whitney rank-sum test. The rest, stress, infarct-zone and remote-zone perfusion values were compared between the two techniques using a paired t-test. The rest, stress infarct-zone and remote-zone perfusion values were compared within the same technique using ANOVA on ranks. $P < 0.05$ was considered statistically significant.

Results

ASL

Example Look-Locker images representing typical image quality obtained with ASL are shown in Figure 1. The black contours that appear at the edges of the LV in Figure 1A–B are due to partial volume effects in pixels containing both magnetization that has been inverted (myocardium) and magnetization that has not been inverted (blood). Supporting Figure S1 (A–H) shows example Look-Locker images obtained after a slice-selective inversion under the conditions of vasodilatory stress and after myocardial infarction. Figure 2 shows example myocardial longitudinal magnetization curves obtained from a mouse at rest (Figure 2A) and during vasodilation (Figure 2B). Figure 2C shows example difference curves and fits to the kinetic model for mice at rest (blue) and with vasodilation (red). A greater difference between the SS and NS curves is easily observed at vasodilation compared to rest. Figure 2D shows an example ASL perfusion map obtained from a mouse at rest.

First-Pass MRI

Figure 3 shows example first-pass images obtained from a mouse at rest acquired using the CS-accelerated sequence. Supporting Figure S1 (I–P) shows example first-pass images obtained under vasodilatory stress and after myocardial infarction. Figure 4A shows example AIF (blue) and TF (red) data and fits obtained from a mouse under resting conditions. Figure 4B shows example TF data and fits obtained at rest (blue) and after vasodilation (red), and Figure 4C shows an example perfusion map obtained from a mouse at rest.

Perfusion values and image quality results

Absolute myocardial perfusion values for ASL and first-pass MRI obtained under the various conditions are shown in Figure 5, and are within the ranges of previously published measurements for MBF in mice at rest (11–13,15–18,25,26) and at stress (11,13,16,18,26,27). The MBF values obtained using first-pass MRI are lower than the values obtained using ASL at rest, stress and in the remote zone (Figure 5A, $p<0.05$), however there was good correlation between the two techniques over the entire range of MBF (Figure 5B). The image quality (IQ) scores were higher for ASL than first-pass for all conditions and both readers (Table 1, $p<0.05$).

Repeatability and between-session coefficient of variability

Bland-Altman plots of between-session repeatability for ASL (Figure 6A) and first-pass MRI (Figure 6B) illustrate the mean difference in perfusion between sessions and are used to compute the RC. The overall RC values (including the rest, stress, infarct and remote data) for each individual technique (ASL and first-pass MRI) were both 55%. For ASL, RC (which is a percentage) increased with decreasing flows (Table 2). For first-pass MRI, RC was fairly similar at rest, stress and in the post-MI infarct and remote zones (Table 2). When comparing ASL and first-pass imaging, we found that RC was much lower with first-pass than ASL in the low blood-flow condition (infarct, $p<0.05$), slightly lower with first-pass compared to ASL in intermediate flows such as rest, and comparable in high MBF conditions (vasodilation).

The overall CV_{bs} (including the rest, stress, infarct and remote data) was $22 \pm 4\%$ for ASL and $14 \pm 2\%$ for first-pass. Similar to RC, the infarct zone CV_{bs} was significantly lower with first-pass as compared to ASL (Table 2, $17 \pm 3\%$ with first pass vs. $46 \pm 9\%$ with ASL, $p<0.05$). CV_{bs} was comparable for the two techniques at rest, stress and in the remote zone (Table 2).

Intra-user variability, Inter-user variability and Inter-animal variability

Bland-Altman plots of intra-user variability and inter-user variability (Figure 7) illustrate the mean difference in perfusion for a single individual analyzing the same data in two different sessions and two different individuals analyzing the same data, respectively. From the Bland-Altman plots, it can be seen that both the intra-user and inter-user variability are consistent under a variety of conditions for ASL, but they increase at higher flows for first-pass MRI.

The overall $CV_{intra-user}$ (including the rest, stress, infarct and remote studies) was $10 \pm 2\%$ for ASL and $11 \pm 1\%$ for first-pass MRI. The $CV_{intra-user}$ was comparable between the two techniques for the rest and remote zone perfusion studies (Table 3). The stress perfusion $CV_{intra-user}$ was significantly lower for ASL compared to first pass (Table 3, $3 \pm 1\%$ vs. $14 \pm 3\%$, $p<0.05$). The infarct zone $CV_{intra-user}$ trended lower for first-pass MRI as compared to ASL (Table 3, $12 \pm 3\%$ vs. $21 \pm 5\%$, $p=0.27$).

The overall $CV_{inter-user}$ (including the rest, stress, infarct and remote data) was $14 \pm 2\%$ for ASL and $21 \pm 2\%$ for first-pass MRI. The $CV_{inter-user}$ was significantly higher for first-pass

MRI compared to ASL at stress (Table 3, $17 \pm 4\%$ vs. $4 \pm 1\%$, $p < 0.05$). The $CV_{\text{inter-user}}$ trended higher for first-pass MRI as compared to ASL at rest (Table 3, $13 \pm 3\%$ vs. $9 \pm 2\%$, $p = 0.17$) and was comparable between the two techniques for the infarct and remote zones.

CV_a was comparable between ASL and first-pass MRI for the rest, stress, infarct and remote zone perfusion studies (Table 3).

Discussion

In this study we have compared ASL and first-pass MRI for the evaluation of myocardial perfusion in preclinical mouse imaging studies. Our comparison included metrics of image quality, repeatability, and variability; and was conducted under varying physiological conditions such as at rest, at stress, and after induction of MI.

Image quality

In this study, image quality was better for ASL than for first-pass MRI under all conditions (rest, stress and post-MI). The reasons for the superior image quality provided by ASL are two-fold. First, since the acquisition for ASL was segmented, where only one spiral interleaf was acquired per heartbeat, there was very little cardiac motion during the image acquisition (which required less than 3ms per interleaf). In contrast, in order to capture the rapid kinetics of a single gadolinium bolus, the first-pass acquisition was not segmented, and image quality was more likely to be degraded by cardiac motion. The times to acquire one AIF image and one TF image were approximately 25ms and 36ms, respectively. These times are fairly short compared to a typical RR interval for a mouse, which is approximately 120ms at rest and 100ms at stress. However, reducing the scan time would further reduce motion artifact and improve image quality. Second, ASL was respiratory gated, and thus data were not acquired outside the respiratory acceptance window. However, in order to capture the rapid kinetics of the gadolinium bolus, respiratory gating could not be employed for first-pass imaging. While we did use a motion-compensated CS reconstruction algorithm, respiratory motion likely caused more artifacts in first-pass imaging than in ASL. In this study, the flip angle for ASL was kept very low (3°) in order to minimize the perturbation to magnetization recovery. However, the low flip angle decreased SNR and sensitivity. Others have previously used higher flip angles and methods which account for magnetization perturbation during perfusion analysis (11,25).

Image acquisition time

Although the image quality of ASL is higher than first-pass, there is a substantial difference in the total acquisition times of the two techniques. While the acquisition time for first-pass imaging was less than a minute, it was approximately 40 minutes for ASL. However, the importance of acquisition time in animal imaging can be very different than in human imaging. Whereas there is an emphasis on short scan times in humans due to healthcare economics and patient comfort, for animal imaging accuracy and precision in quantifying physiological parameters may take precedence. By spending more time per animal to improve the accuracy and precision of a measurement, it may be possible to reduce the total number of animals needed to test a hypothesis and complete a study. In this way, it may be

more economical and ethical to use longer acquisitions. In the present study, acquisition parameters were determined independently for ASL and first-pass MRI in order to achieve good image quality so that the images would be well-suited for quantitative analyses. While first-pass MRI will always be faster than ASL, in the future we will shorten the ASL scans by using a segmented acquisition where more spiral interleaves are acquired in each segment and, possibly, by using higher acceleration rates and lower spatial resolution. A recent study by Troalen *et al.* (25) used a new ASL technique in mice which has an 8-minute acquisition time. Another study by Campbell *et al.* (27) used a segmented Look-Locker ASL technique in mice with a reduced acquisition time of 15 minutes for both slice-selective and non-selective scans.

Perfusion values

Although there is reasonable agreement between the perfusion values obtained using the two techniques (Figure 5), the absolute perfusion values obtained using first-pass MRI are lower than those obtained using ASL. The absolute discrepancy between the techniques was observed to increase as flow increased. One potential reason could be the reduced image quality of the first-pass technique under conditions with higher heart rates such as under stress with Regadenoson. Additionally, while numerous steps and assumptions are required to estimate gadolinium concentration and perfusion in the analysis of first-pass MRI, perfusion analysis of ASL is simpler. The simplicity of ASL analysis suggests that perfusion values by ASL may be more accurate.

Repeatability

The RC indicates the change in perfusion between different treatments or groups that is required to detect differences above systematic errors. With first-pass MRI, the RC is approximately 30–35%. This is consistent with our recent study which showed that stress perfusion was reduced by almost 30% in wild type C57Bl/6 mice fed a high-fat diet as compared to wild type mice on a standard chow diet (16).

The RC for infarcted tissue was much greater using ASL compared to first-pass MRI. This is likely because ASL has lower perfusion sensitivity than first-pass MRI, and the infarct zone has low perfusion. Perfusion sensitivity is the change in MRI signal due to blood flow. Because ASL has lower perfusion sensitivity (Appendix 2) and perfusion is relatively low in the infarcted region, the ASL signal from this area is only slightly greater than the noise. This low signal-to-noise ratio likely leads to poor RC.

Campbell *et al.* (27) studied the repeatability of segmented ASL in mice at rest. However, their study used a higher isoflurane dose than our study (1.6% vs. 1.2%). Because isoflurane is a potent vasodilator (11), the mean perfusion values (approximately 12 ml/g/min) obtained in their study were comparable to the stress perfusion values obtained in the present study. Our overall RC is similar to their result (55%). However, the RC specifically for the high flow regime is lower with both of our techniques as compared to their reported value (41% vs. 55% with our ASL method, and 31% vs. 55% with our first-pass method).

Variability

The CV_{bs} takes into account differences associated with the technique such as repositioning the mouse, reshimming, replacing the ECG leads and i.v. line, and physiological differences in the mouse between the two sessions. The CV_{bs} indicates the variation to expect when imaging the same set of animals over time. Similar to the results obtained with RC, CV_{bs} was significantly lower with first-pass MRI as compared to ASL in low MBF conditions (infarct). This is probably due to low perfusion sensitivity with ASL at low MBF. Compared to the Campbell study (27), the CV_{bs} values for stress perfusion studies obtained for both techniques in this study are slightly lower than their reported value (13% vs. 17% with ASL and 14% vs. 17% with first-pass MRI). Van Nierop *et. al.* (17) reported between-session CV with a dual – bolus first-pass technique in mice at rest. Our first-pass CV_{bs} at rest is slightly higher than their reported value (11% vs. 6%).

The $CV_{intra-user}$ takes into account variation associated with the analysis such as redrawing the ROIs and the various steps in reanalyzing the perfusion datasets. The stress perfusion $CV_{intra-user}$ was significantly lower for ASL as compared to first-pass MRI, probably due to the higher image quality of stress perfusion images with ASL. The $CV_{intra-user}$ trended lower with first-pass MRI as compared to ASL for the infarct studies, probably due to higher perfusion sensitivity of first-pass MRI at low blood-flow conditions.

The $CV_{inter-user}$ was lower for ASL than for first-pass MRI under all conditions. This is likely due to the low degree of user interaction in ASL analysis as opposed to first-pass perfusion analysis. There are multiple steps in the first-pass analysis that involve user interaction such as drawing the ROIs for the LV blood pool and myocardium and adjusting them at all time points, fitting a gamma-variate function to the AIF data, and determining the end time of first-pass kinetics. In comparison, ASL analysis requires user interaction only when contouring the LV blood pool and LV myocardium at a single time point.

The CV_a indicates the variation to expect between the animals and this can be useful in deciding the sample sizes while planning an experiment. The CV_a values were comparable to previously reported values as calculated using the means and standard deviations (11–13, 15–18, 25, 27). Prior studies reported CV_a of 8–27% (11–13, 15–18, 25, 27) at rest and stress and our reported values (16–18% with first-pass MRI and 19% with ASL) are within this range. A prior study (15) reported CV_a of 67% in the infarct zone with permanent ligation and our infarct-zone CV_a values are lower than their reported value for both techniques (27% with first-pass MRI and 44% with ASL).

A limitation of this study is that the active length of the RF coil was 70 mm, which is not long enough to cover the entire mouse body. Hence perfusion estimated by ASL may be underestimated since a fraction of the blood will not be inverted by the nonselective inversion pulse. Another limitation of this study is that we used different spatial resolutions for ASL and first-pass acquisitions. The LV wall in mice has a width of approximately 1 mm, which corresponded to approximately 3–4 pixels using the spatial resolution achieved by first-pass MRI. Using the spatial resolution of our ASL protocol, we had approximately 7–10 pixels across the LV wall. While 7–10 pixels across the LV wall is preferred, the higher

resolution of ASL and its lower perfusion sensitivity led to noisier perfusion maps for ASL as compared to first-pass MRI (Figures 2D and 4D).

Conclusions

Although the overall repeatability and data variability are comparable between ASL and first-pass MRI, each technique has its own advantages and disadvantages, and depending on the situation, one technique may be more suitable than the other. For low MBF conditions such as infarct imaging, first-pass MRI is more suitable for myocardial perfusion imaging in mice due to better repeatability (lower RC and CV_{bs}) and variability (lower $CV_{intra-user}$). Additionally, accounting for the issue of overall scan time, first-pass MRI in mice integrates well into a comprehensive MRI protocol due to its short acquisition time. On the other hand, at higher MBFs such as stress perfusion imaging, ASL may be more suitable than first-pass MRI due to its superior image quality and reduced inter-user variability. Furthermore, due to the ability to employ segmented acquisitions and respiratory gating with ASL, it is straightforward to achieve higher spatial resolution with ASL compared to first-pass MRI. The advantages of ASL are amplified at stress where heart rates can be as high as 650–700 bpm and respiratory rates are also increased. Additionally, for circumstances where the stress agent can be injected into the peritoneum, the imaging protocol for ASL would be much simpler as it would not require any i.v. lines. In that case, for imaging studies which require serial perfusion imaging in the same animals over time, ASL could be advantageous since repetitive placement of i.v. lines could be difficult. The findings of the present study, which accounted for many competing factors, should prove useful for planning future studies that use myocardial perfusion imaging to investigate underlying mechanisms and therapies for treating perfusion abnormalities in various mouse models of cardiovascular disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix 1

ASL Quantitative Perfusion Analysis

Multi-inversion-time ASL data were analyzed using a kinetic model based on the formalism developed by Hrabe *et al.* (28) which we modified to use a gamma-variate function to model the distribution of transit times for arterial water flowing through the coronary arterial circulation. The gamma-variate distribution has for many years been used to model blood flow through vascular beds (29–31), and was recently applied to ASL of the heart (32) and brain (33).

The ASL approach employed in this study used a FAIR preparation scheme, where the tagged image was acquired after application of a slice-selective inversion, and the control image was acquired after application of a non-selective inversion. The modified Bloch equations for tissue longitudinal magnetization in the presence of perfusion (34) after a slice-selective (M_{SS}) and non-selective (M_{NS}) inversion can be written as:

$$\frac{dM_{SS}}{dt} = \frac{M_0 - M_{SS}(t)}{T_1} + fM_{SS}^A(t) - \frac{f}{\lambda}M_{SS}(t), M_{SS}(0)=0 \quad (\text{Eq. 2})$$

$$\frac{dM_{NS}}{dt} = \frac{M_0 - M_{NS}(t)}{T_1} + fM_{NS}^A(t) - \frac{f}{\lambda}M_{NS}(t), M_{NS}(0)=0 \quad (\text{Eq. 3})$$

where M_0 is the equilibrium tissue magnetization, f is the flow in s^{-1} , λ is the blood/tissue water partition coefficient and T_1 is the relaxation time constant of myocardium. $M_{SS}^A(t)$ and $M_{NS}^A(t)$ are the arterial blood magnetizations after slice-selective and non-selective inversions respectively.

If we define the difference function as $D(t) = M_{SS}(t) - M_{NS}(t)$ (28), then the differential equation for the difference function can be written as follows:

$$\frac{dD(t)}{dt} = \frac{dM_{SS}}{dt} - \frac{dM_{NS}}{dt}, D(0)=0$$

Using Equations 2 and 3, this can then be rewritten : as

$$\frac{dD(t)}{dt} = -D(t) \left(\frac{1}{T_1} + \frac{f}{\lambda} \right) + f \left(M_{SS}^A(t) - M_{NS}^A(t) \right)$$

$$\text{If } \frac{1}{T_{1app}} = \left(\frac{1}{T_1} + \frac{f}{\lambda} \right) \text{ and } D^A(t) = f \left(M_{SS}^A(t) - M_{NS}^A(t) \right), \text{ then}$$

$$\frac{dD(t)}{dt} + \frac{D(t)}{T_{1app}} = D^A(t) \quad (\text{Eq. 4})$$

Where T_{1app} is the apparent T_1 of the tissue.

For the SS scan, the inflowing arterial blood is not inverted, while for the NS scan the inflowing arterial blood is inverted. Thus $D^A(t)$ can be solved as follows:

$$D^A(t) = 2fM_B^0 W(t) e^{-t/T_{1B}} \quad (\text{Eq. 5})$$

where M_B^0 is the proton density of blood, $W(t)$ describes the arterial blood input function and T_{1B} is the T_1 relaxation time constant for blood. If we assume the impulse response is a gamma-variate function, then

$$h(t) = (t - t_{\text{delay}})^{\alpha} e^{-\frac{(t - t_{\text{delay}})}{\beta}} \text{ and } W(t) = \int_{\tau=0}^t h(\tau) d\tau$$

where $h(t)$ is the blood transit time distribution, α determines the gamma-variate upslope, β determines the decay of $h(t)$ and t_{delay} is the transit delay. Then the gamma-variate based solution to the ASL equation can be solved using Equations 4 and 5 as:

$$D(t) = 2fM_B^0 W(t) e^{-\frac{t}{T_{1B}}} * e^{-\frac{t}{T_{1\text{app}}}}$$

where $*$ stands for convolution operation.

Appendix 2

Perfusion sensitivity

We define perfusion sensitivity as the change in MRI signal for a given amount of blood flow. To help explain why first-pass MRI performed better than ASL at low MBF even though image quality was better for ASL, we estimated the perfusion sensitivities for these two techniques under the specific experimental conditions of this study. In general terms, because first-pass MRI uses gadolinium as a T1-shortening contrast agent to amplify the effects of perfusion on the signal and ASL does not use this amplification method, it is clear that first-pass MRI will have greater perfusion sensitivity than ASL. To quantify the perfusion sensitivities for each technique, we used the Fermi function model and Bloch equation simulations to estimate signals for first-pass imaging, and we used the gamma-variate based ASL kinetic model to estimate ASL signals. Using these models, we estimated ASL and first-pass MRI signals for MBF values of 1, 5.8 and 13.1 ml/g/min (representing the range of MBFs measured in the mouse heart in this study), using the specific pulse sequence parameters and gadolinium dose employed in this study. Perfusion sensitivity for first-pass MRI was higher than ASL by approximately 75%, 23%, and 27% for MBFs in the low, intermediate, and high regimes, respectively (Supporting Figure S2). The greater perfusion sensitivity gained through the use of gadolinium is an advantage of first-pass MRI compared to ASL, even under conditions specific to mouse heart imaging where the time available for saturation recovery is compressed compared to human imaging.

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Figure 1.

(A–D): Example CS-accelerated Look-Locker ASL images obtained from a mouse at rest after a slice-selective (SS) inversion and reconstructed using BLOSM. (E–H): Example CS-accelerated Look-Locker ASL images obtained from a mouse at rest after a non-selective (NS) inversion and reconstructed using BLOSM.

Figure 2.

(A): Representative myocardial longitudinal magnetization curves obtained from a mouse at rest after a non-selective (blue circles) and slice-selective (red circles) inversion. (B): Representative myocardial longitudinal magnetization curves obtained from a mouse during vasodilation after a non-selective (blue circles) and slice-selective (red circles) inversion. The slice-selective T1 is reduced during vasodilation compared to rest. (C): Example difference data and fits to the kinetic ASL model obtained from a mouse at rest (blue) and at stress (red). (D): Example pixel-by-pixel perfusion map obtained using ASL from a mouse at rest.

Figure 3.
(A–D): Example CS-accelerated dual-contrast first-pass MR images obtained from a mouse at rest and reconstructed using BLOSM.

Figure 4.

(A): Example first-pass MRI arterial input function (AIF, blue) and tissue function (TF, red) data and fits obtained from a mouse at rest. (B): Example TF data and fits obtained from a mouse at rest (blue) and at stress (red). (C): Example pixel-by-pixel perfusion map obtained using first-pass MRI from a mouse at rest.

Figure 5.

(A): Perfusion results obtained in wild-type mice at rest, stress, in the infarct zone and in the remote zone using both the ASL and first-pass MRI techniques (*p < 0.05 vs. first-pass, #p < 0.05 vs. infarct using same technique, \$p < 0.05 vs. rest, infarct and remote using the same technique). (B): Orthogonal regression plot showing the correlation of perfusion measurements made using the first-pass and ASL techniques.

Figure 6.

Bland-Altman plots showing the repeatability of perfusion measurements obtained using the two perfusion imaging techniques: ASL (A) and first-pass MRI (B).

Figure 7.

Intra-user variability (A–B) and inter-user variability (C–D) of ASL (A,C) and first-pass MRI (B,D).

Table 1

Image quality scores for ASL and first-pass MRI.

		First-pass MRI	ASL
Reader 1	Rest	3.07 ± 0.20	$3.86 \pm 0.21^*$
	Stress	2.14 ± 0.18	$3.71 \pm 0.19^*$
	Infarct	3.36 ± 0.23	$4.00 \pm 0.28^*$
Reader 2	Rest	3.36 ± 0.23	4.00 ± 0.28
	Stress	2.43 ± 0.23	$3.5 \pm 0.23^*$
	Infarct	3.21 ± 0.24	$4.14 \pm 0.21^*$

*
p < 0.05 vs. First-pass MRI

Table 2

Repeatability and between-session coefficient of variability for ASL and first-pass MRI.

		ASL	First-pass MRI
Repeatability Coefficient (RC) (%)	Rest	62	36
	Stress	41	31
	Infarct	138	56
	Remote	44	40
Between-session CV (CV_{bs}) (%)	Rest	18.55 ± 4.57	10.77 ± 2.18
	Stress	12.66 ± 3.34	14.15 ± 3.59
	Infarct	46.28 ± 9.33	$17.03 \pm 3.44^*$
	Remote	11.32 ± 2.90	14.50 ± 3.70

* $p < 0.05$ vs. ASL

Table 3

Intra-user, inter-user and inter-animal variability for ASL and first-pass MRI.

		ASL	First-pass MRI
Intra-user CV ($CV_{\text{intra-user}}$) (%)	Rest	7.21 ± 1.51	7.56 ± 1.34
	Stress	$2.82 \pm 0.58^*$	14.33 ± 3.03
	Infarct	21.30 ± 5.25	12.12 ± 2.97
	Remote	8.96 ± 2.25	9.67 ± 1.78
Inter-user CV ($CV_{\text{inter-user}}$) (%)	Rest	8.95 ± 1.51	13.30 ± 3.22
	Stress	$3.69 \pm 0.75^*$	17.16 ± 4.21
	Infarct	24.55 ± 6.32	28.68 ± 5.21
	Remote	15.89 ± 5.97	16.70 ± 3.83
Inter-animal variability (CV_a) (%)	Rest	18.34 ± 0.85	15.55 ± 3.60
	Stress	19.00 ± 2.59	18.31 ± 2.54
	Infarct	44.01 ± 8.58	26.10 ± 2.68
	Remote	20.47 ± 4.08	27.35 ± 3.59

*
p < 0.05 vs. First-pass MRI