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Accelerated Dual-contrast First-pass Perfusion MRI of the Mouse Heart: Development and Application to Diet-induced Obese Mice

Nivedita K. Naresh, M.Eng.¹, Xiao Chen, B.S.¹, Rene J. Roy, B.S.², Patrick F. Antkowiak, Ph.D.¹, Brian H. Annex, M.D.³, and Frederick H. Epstein, Ph.D.^{1,2}

¹Department of Biomedical Engineering, University of Virginia, Charlottesville, Virginia, USA

²Department of Radiology, University of Virginia, Charlottesville, Virginia, USA

³Department of Cardiovascular Medicine, University of Virginia, Charlottesville, Virginia, USA

Abstract

Background—Gene-modified mice may be used to elucidate molecular mechanisms underlying abnormal myocardial blood flow (MBF). We sought to develop a quantitative myocardial perfusion imaging technique for mice and to test the hypothesis that myocardial perfusion reserve (MPR) is reduced in a mouse model of diet-induced obesity (DIO).

Methods—A dual-contrast saturation-recovery sequence with k_y -t undersampling and a motion-compensated compressed sensing reconstruction algorithm was developed for first-pass MRI on a small-bore 7T system. Control mice were imaged at rest and with the vasodilators ATL313 and Regadenoson (n=6 each). In addition, we imaged mice fed a high-fat diet (HFD) for 24 weeks.

Results—In control mice, MBF was 5.7 ± 0.8 ml/g/min at rest and it increased to 11.8 ± 0.6 ml/g/min with ATL313 and to 10.4 ± 0.3 ml/g/min with Regadenoson. In HFD mice we detected normal resting MBF (5.6 ± 0.4 vs. 5.0 ± 0.3 on control diet), low MBF at stress (7.7 ± 0.4 vs. 10.4 ± 0.3 on control diet, $p < 0.05$), and reduced MPR (1.4 ± 0.2 vs. 2.0 ± 0.3 on control diet, $p < 0.05$).

Conclusions—Accelerated dual-contrast first-pass MRI with motion-compensated compressed sensing provides spatiotemporal resolution suitable for measuring MBF in free-breathing mice, and detected reduced MPR in DIO mice. These techniques may be used to study molecular mechanisms that underlie abnormal myocardial perfusion.

Introduction

Assessing myocardial perfusion or perfusion reserve (MPR) is of value in the diagnosis of ischemic heart disease, and impaired MPR has been shown to be prognostic of adverse cardiovascular outcomes (1). Furthermore, myocardial perfusion or perfusion reserve may be reduced due to obstructive epicardial atherosclerosis or due to microvascular disease.

Correspondence to: Frederick H. Epstein, Ph.D. Professor of Biomedical Engineering and Radiology, 415 Lane Road, Charlottesville, VA 22908, fred.epstein@virginia.edu, Phone: 434-924-0270, Fax: 434-982-3870.

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MPR is defined as the ratio of myocardial blood flow at maximal hyperemia to myocardial blood flow at rest. In patient populations of diabetics, women, and individuals with the metabolic syndrome, the mechanisms underlying reduced perfusion or MPR are not completely understood (2–4). Research using transgenic and knockout mice has provided tremendous insights into the genetic, molecular, and cellular mechanisms underlying every major cardiovascular disease, and has contributed extensively to the development of new medical therapies (5–8). Quantitative myocardial perfusion imaging in gene-modified mice may be used to elucidate molecular mechanisms underlying insufficient perfusion and to assess the efficacy of experimental therapies. Currently, quantitative myocardial perfusion imaging in mice can be performed using arterial spin labeling (ASL) MRI (9–13), however, ASL requires long acquisition times.

First-pass gadolinium (Gd)-enhanced MRI is a well-established technique to quantify MBF and MPR in humans and it has only been recently investigated in mice (14–18). First-pass MRI in mice is challenging due to the rapid heart rate and small size of the mouse heart, and because image acquisition occurs during free breathing, which can lead to image artifacts. Recent MRI acceleration techniques have made first-pass imaging in mice possible. Quantitative first-pass MRI relies on the accurate measurement of both the myocardial tissue function (TF) and the arterial input function (AIF), which is usually measured in the left ventricular (LV) blood pool. Quantitative first-pass MRI in humans is often performed using either a dual-bolus technique or a dual-contrast technique. A dual-bolus technique uses a low dose injection of the contrast agent to measure the AIF followed by a high dose contrast-agent injection to measure the myocardial tissue function (TF) (19, 20). On the other hand, a dual-contrast acquisition uses a single high dose contrast-agent injection to measure both the AIF using a short saturation delay and the TF using a longer saturation delay (21). A dual-contrast acquisition simplifies the procedure (one Gd injection) and may provide more accurate results than the dual-bolus method because the AIF and TF are acquired under identical conditions.

Our goals for this study were to develop a dual-contrast first-pass sequence for mice and to use these methods to test the hypothesis that C57Bl/6 mice fed a high-fat diet (HFD) for 24 weeks have reduced MPR. This diet-induced obesity (DIO) model was recently shown to recapitulate many hallmarks of human diabetic cardiomyopathy after 8 months on diet, including obesity, hyperglycemia, insulin resistance, and hyperinsulinemia, all in the absence of epicardial coronary artery disease (22). For the first time, we sought to establish another hallmark of human diabetic cardiomyopathy, namely reduced MPR, in these mice.

Methods

MRI Hardware and First-pass Pulse Sequence

MRI was performed on a 7T Clinscan system (Bruker, Ettlingen, Germany) equipped with actively shielded gradients with a full strength of 650 mT/m and a slew rate of 6666 mT/m/ms. Additional equipment included a 30 mm diameter birdcage RF coil and an MR-compatible physiological monitoring and gating system for mice (SA Instruments, Inc., Stony Brook, NY).

A dual-contrast saturation-recovery Cartesian sequence with a gradient-echo readout and k_y - and time domain (k_y -t) undersampling was implemented. The acquisition scheme is illustrated in Figure 1. The saturation pulse was chosen to be an adiabatic BIR4 pulse with a duration of 9 ms, in order to achieve uniform saturation (23). Two short-axis slices (slice gap = 0.2 mm) were acquired within each cardiac cycle, one to obtain the AIF and the other to obtain the TF. Respiratory gating was not applied. For both slices, the center of k-space was acquired at the Nyquist sampling rate while the higher spatial frequencies were randomly undersampled in the k_y -t domain. This undersampling scheme was employed in order to facilitate image reconstruction using compressed sensing (24). The AIF slice was undersampled at rate six while the TF slice was undersampled at rate four. These acceleration rates of 6 and 4, respectively, have previously been shown to provide good image quality for human first-pass images reconstructed using a motion-compensated compressed sensing algorithm, even when respiratory motion occurs (25). Undersampled proton density images were acquired toward the end of the acquisition in order to normalize signal intensities for perfusion analysis. Other relevant imaging parameters were: TE/TR = 1.2/2.1 ms, FOV = 25.6×18 –20 mm², phase FOV = 72%, percent sampling = 80%, image resolution = 200 μm^2 , matrix = 128×74 –104, excitation flip angle = 15°, slice thickness = 1 mm, AIF saturation delay = 15 ms, TF saturation delay = 57–74 ms, AIF image acquisition time = 25–34 ms/image and TF image acquisition time = 36–53 ms/image. 12–16 phase-encode lines were acquired for each AIF image and 17–25 lines were acquired for each TF image. For the AIF images centric k-space filling was used to enable a short saturation delay, while for the TF images linear k-space filling was used.

Compressed Sensing Reconstruction

A motion-compensated compressed-sensing technique called Block LOw-rank Sparsity with Motion-guidance (BLOSM) was recently developed to reconstruct undersampled dynamic images, including images acquired during breathing (25). This method utilizes the concept of spatiotemporal low rank sparsity, as used in the k-t Sparsity and Low-Rank (k-t SLR) technique (26). In BLOSM, the image is divided into regions (or blocks), motion tracking of the blocks is embedded within the iterative algorithm, and regional low-rank sparsity is applied to the motion-tracked blocks. BLOSM achieved very good results in human first-pass images, including those with respiratory motion (25). Because first-pass MRI in mice occurs during breathing, BLOSM was chosen to reconstruct undersampled first-pass data in this context.

Experimental Design

The experimental design was divided into two parts: 1) evaluation of the sequence over a range of vasodilator doses and using two different vasodilator agents using wild-type C57Bl/6 mice fed a standard chow diet, and 2) use of the sequence to test the hypothesis that MPR is reduced in DIO mice. For the first part, 20–24 week old wild type C57Bl/6 mice were imaged using the dual-contrast first-pass sequence at rest ($n = 6$) and with the vasodilators Regadenoson ($n = 6$) (Lexiscan, Astellas Pharmis, 0.1 $\mu\text{g/g}$ body weight) and ATL313 (separate group, $n = 6$) (Adenosine Therapeutics, 0.025 $\mu\text{g/g}$ body weight). In addition to these doses, 12 week old wild type mice ($n = 5$) were imaged with different doses of Regadenoson (0.01, 0.02 and 0.05 $\mu\text{g/g}$ body weight) in order to test whether first-

pass MRI could detect incremental increases in MBF. The different doses of Regadenoson were administered in different imaging sessions that were performed on separate days. The reason for this is that the terminal half-life of Regadenoson is very long (33–108 minutes) (27). Thus the wait time for the effect of Regadenoson to wear off would be long, thereby making the imaging session too long. Finally, C57Bl/6 mice fed a HFD (Diet # D12492, Research Diets, Inc., New Brunswick, NJ) were studied. Specifically, DIO mice fed a HFD for 24 weeks (n = 6) and age-matched wild type C57Bl/6 mice (n = 5) fed standard chow diet were imaged at rest and with Regadenoson (0.1 µg/g body weight) using the dual-contrast sequence. DIO mice were started on the diet at 6 weeks of age and the imaging was performed after continuing the diet for 24 weeks. Only DIO mice that were glucose intolerant were selected for imaging. For the glucose-tolerance test (GTT) (28), mice were injected intraperitoneously with 1 g/kg glucose in milli-Q water after overnight fasting. A tail vein blood sample was taken before and at 10, 30, 60, 90 and 120 minutes post-injection of the glucose solution for determination of blood glucose. The blood glucose values were then plotted versus time and the area under the curve was calculated to evaluate glucose tolerance.

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 Gd-DTPA (Magnevist, 0.1 mM/kg body weight) and Regadenoson, while ATL313 was delivered by an intraperitoneal (IP) bolus injection. Mice were positioned supine in the magnet and body temperature was maintained at 36 ± 0.5 °C using thermostated water. Anesthesia was maintained using 1.25% isoflurane in O₂ 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).

MRI Protocol

In all mice, localizer cine imaging was performed to select a mid-ventricular short axis slice. For first-pass imaging, the CS-accelerated dual-contrast first-pass sequence was used as described above. 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 in order to capture first-pass kinetics. For the stress-perfusion acquisition, Regadenoson was injected through a tail vein catheter and 10 minutes later first-pass imaging was performed while injecting Magnevist through the same tail vein catheter. We waited for 10 minutes after the injection of the stress agent for the heart rate to stabilize before injecting Magnevist and performing the first-pass acquisition.

Perfusion analysis

All data analysis was based on Fermi function deconvolution, implemented in MATLAB (Mathworks, Inc). To generate signal intensity vs. time curves, one region of interest (ROI) was placed in the LV blood pool in the images with the short saturation delay to obtain the

AIF, while the TF was generated using a ROI covering the entire myocardial tissue in the images acquired using the longer saturation delay. These signal intensities were normalized by the signal intensities of the proton density images and thereafter were converted to T1 values using the methods described by Axel *et al* (29). Since the accuracy of this method is poor at long T1 values (29), prior ASL measurements were used to fix pre-contrast T1 values for the blood (1.55 s) and for the myocardium (1.45 s). Precontrast T1 was measured in 6 wild type mice (separate group) using a Look-Locker sequence with a 3° excitation flip angle (13, 30). The T1-relaxation measurements were fit to this equation:

$M_z = M_0 \exp(-t/T_1)$. Precontrast blood T1 was found to be 1.55 ± 0.04 s which is in agreement with prior studies (11, 13) while the precontrast myocardial T1 was found to be 1.45 ± 0.02 s, which is also in agreement with prior studies (13, 30). Relaxivity was assumed to be 3.8L/mmol.s (31) which was used to convert T1 values to gadolinium concentrations. Absolute MBF was calculated from the LV blood pool and myocardial tissue gadolinium concentration-time curves (AIF and TF) using Fermi constrained deconvolution (32). To demonstrate the ability of the CS-accelerated dual-contrast sequence to quantify regional perfusions, the myocardium was divided into 6 sectors which were identified clockwise starting from the RV insertion point as: anterior, anterolateral, inferolateral, inferior, inferoseptal, and antero-septal. Regional tissue functions were obtained by placing ROIs in these sectors and regional perfusions were quantified using Fermi function deconvolution.

Statistical analysis

All MBF values are expressed as mean $\pm$ standard error. For comparing rest and stress perfusion in wild type and DIO mice the student t-test was used. One-way analysis of variance was used to compare perfusions with different stress doses for the dose-response. Two-way analysis of variance was used to compare rest and stress perfusions within the same sector and between the different sectors. $P < 0.05$ was considered to be statistically significant.

Results

Evaluation of the sequence

Example AIF images obtained from a free-breathing mouse at rest using a short saturation delay are shown in Figure 2(A–F), and representative TF images from the same mouse obtained using a longer saturation delay are shown in Figure 2(G–L). Figure 2(A–C) shows example AIF images undersampled at rate 6 and reconstructed using 2D Fast Fourier Transform (FFT), while Figure 2(D–F) shows these AIF images reconstructed using the BLOSM technique. Figure 2(G–I) shows example TF images undersampled at rate 4 and reconstructed using 2D FFT, and Figure 2(J–L) shows these images reconstructed using the BLOSM technique (25). Since the AIF uses a short saturation delay, the images are acquired during systole, when the LV blood pool is only a few pixels wide (especially after vasodilation). Accordingly, a high-resolution AIF acquisition is necessary in order to resolve the LV blood pool. Figure 3A shows representative AIF and TF data and fits from Fermi function deconvolution obtained from a mouse at rest while Figure 3B shows TF data and fits obtained from mice at rest and after vasodilation. Average peak gadolinium concentration in the LV blood pool was found to be 2.3 ± 0.2 mM at rest and 2.9 ± 0.3 mM

after vasodilation for the 6 mice that were studied. Average peak gadolinium concentration in the myocardial tissue was found to be 0.4 ± 0.03 mM at rest and 0.6 ± 0.09 mM after vasodilation. The first-pass kinetics in mice occurred rapidly, over approximately 2–3 s. The average mouse heart rate in our study was 455 ± 15 bpm at rest and 563 ± 3 bpm with vasodilation (R-R interval ~ 120 ms at rest and 100 ms with vasodilation). The average breathing rate in the mice was 188 ± 19 breaths/min.

Perfusion was measured in wild-type mice at rest and with the vasodilators ATL313 (0.025 $\mu\text{g/g}$ body weight) and Regadenoson (0.1 $\mu\text{g/g}$ body weight), and these results are summarized in Figure 4. Mean baseline perfusion was found to be 5.7 ± 0.8 ml/g/min and it increased to 11.8 ± 0.6 ml/g/min with ATL313 ($p < 0.05$ vs. rest) and to 10.4 ± 0.3 ml/g/min with Regadenoson ($p < 0.05$ vs. rest). Perfusion reserve was found to be 2.2 with ATL313 and 2.0 ± 0.3 with Regadenoson. Since separate groups of mice were used for rest and ATL313 studies, there is no standard error value reported for perfusion reserve with ATL313. We were also able to detect incremental changes in perfusion using incremental doses of Regadenoson, as shown in Figure 5. In this group of wild type C57Bl/6 mice, baseline perfusion was 6.1 ± 0.5 ml/g/min. Perfusion increased to 7.8 ± 0.4 ml/g/min with 0.01 $\mu\text{g/g}$ body weight dose of Regadenoson, which then increased to 9.8 ± 0.7 ml/g/min with 0.02 $\mu\text{g/g}$ body weight dose of Regadenoson ($p < 0.05$ vs. rest) and thereafter to 11.7 ± 0.3 ml/g/min with 0.05 $\mu\text{g/g}$ body weight dose of Regadenoson ($p < 0.05$ vs. rest, $p < 0.05$ vs. 0.01 $\mu\text{g/g}$). With 0.1 $\mu\text{g/g}$ body weight dose of Regadenoson, MBF did not increase further, and was measured as 10.8 ± 0.9 ml/g/min ($p < 0.05$ vs. rest, $p < 0.05$ vs. 0.01 $\mu\text{g/g}$).

To demonstrate the advantage of acquiring a separate AIF acquisition with a short saturation delay when using a standard dose of gadolinium (as used in this study), Figure 6 compares LV blood pool gadolinium concentration estimates obtained using images acquired with a short (10 ms) and a long saturation delay (57 ms). These data show that with a Gd-DTPA dose of 0.1 mmol/kg body weight, the AIF obtained with a long saturation delay of 57 ms markedly underestimates the peak concentration. Table 1 compares calculated perfusion values at rest and with Regadenoson (0.1 $\mu\text{g/g}$ body weight) estimated using AIFs measured from images with a short and a long saturation delay. These results demonstrate that perfusion is overestimated when the AIF is obtained using images acquired with a long saturation delay. The error in the estimate of the AIF calculated using the images acquired with a long saturation delay may be due to signal errors arising from high blood velocities that occur in certain diastolic phases (33). This blood motion can cause dephasing during the echo time (34), leading to signal loss and lower AIF estimates. Placing the AIF acquisition near end systole provides less sensitivity to signal dephasing.

To demonstrate the ability of the CS-accelerated dual-contrast sequence to quantify regional perfusions, Figure 7 shows example regional TF curves obtained from mice at rest and after vasodilation with Regadenoson. Table 2 shows the average regional perfusions that were obtained in the mice at rest and after vasodilation. There were no significant differences in the rest and stress perfusion values between the different sectors. Stress perfusion was significantly increased within the same sector ($p < 0.05$ vs. rest).

Myocardial perfusion reserve in DIO mice

Using the dual-contrast first-pass sequence with BLOSM reconstruction, we were able to detect reduced MPR in DIO mice fed a HFD for 24 weeks. These results are summarized in Figure 8. Baseline perfusion in DIO mice was found to be 5.6 ± 0.4 ml/g/min and this was similar to the baseline perfusion in wild type mice (5.7 ± 0.8 ml/g/min). Stress perfusion was reduced ($p < 0.05$) in DIO mice (7.7 ± 0.4 ml/g/min) as compared to the wild type mice (10.4 ± 0.3 ml/g/min). Thus, MPR was reduced in DIO mice due to reduced stress perfusion. Specifically, MPR was 2.0 ± 0.3 in wild type mice and 1.4 ± 0.2 in DIO mice ($p < 0.05$).

Discussion

In this study, we developed a dual-contrast first-pass perfusion MRI method for mice, which allowed sampling of both the AIF and TF within every R-R interval. This method employed a CS-accelerated dual-contrast gradient-echo pulse sequence to acquire undersampled first-pass images and the motion-compensated BLOSM CS reconstruction technique to reconstruct the undersampled free-breathing data in the presence of respiratory motion (25). Using this technique, we measured MBF in mice at rest and with two vasodilators, ATL313 and Regadenoson. We also detected increments in perfusion with increasing doses of Regadenoson and obtained a dose-response curve. Using this method, for the first time we measured a reduction in MPR in DIO mice.

The dual-contrast first-pass MRI sequence enables acquisition of both the AIF and TF using a single gadolinium injection in mice. There are several challenges to performing first-pass imaging in mice. The average mouse heart rate in our study was 455 ± 15 bpm at rest and 563 ± 3 bpm with vasodilation (R-R interval ~ 120 ms at rest and 100 ms with vasodilation). Using a fast saturation-recovery gradient-echo sequence with rectangular FOV and acceleration rates of 6 and 4 for the AIF and TF respectively, the total imaging time was 80 ms/R-R interval. This protocol enabled us to sample both the AIF and the TF every R-R interval, both at rest and with vasodilation. The second challenge was the small size of the mouse heart. The dual-contrast first-pass images were acquired with an in-plane resolution of $200 \mu\text{m}^2$, which enabled us to clearly resolve the LV blood pool and myocardium. The third challenge was the free breathing of the mice, which can introduce artifacts in images. Since mice have a fast systemic circulation time of just a few seconds, it is essential to sample the AIF and TF every heart beat and hence respiratory gating cannot be used. In order to reduce respiratory motion artifacts, we used a recently developed CS reconstruction technique called BLOSM. This technique uses motion tracking to guide the CS sparsity transform and compensates for respiratory motion (25).

In this study, we evaluated the sequence at rest and with two vasodilators, ATL313 and Regadenoson. Regadenoson is an A_{2A} receptor agonist that is used clinically. This agent has been shown to have fewer side effects as compared to adenosine and it can be injected as a bolus before contrast agent administration (35). Our results show the feasibility of using this agent for stress first-pass MRI in preclinical studies. The MBF values obtained at rest and with ATL313 agree closely with our prior ASL data (36). Both ATL313 and Regadenoson provided comparable perfusion values during vasodilation. The peak concentration of Gd-DTPA in the LV blood pool with 0.1 mmol/L/kg body weight dose

(bolus concentration = 50mM) was 2.3 ± 0.2 mM. This concentration is similar to the concentration obtained by Nierop *et al.* (1.9 mM) (18), who used a dual-bolus technique in mice with a full bolus concentration of 40 mM. Using the dual-contrast sequence with different doses of Regadenoson, we were able to obtain a dose-response curve. Thus, using this dual-contrast sequence, we were able to measure incremental increases in MBF with incremental doses of Regadenoson until it plateaued at approximately 11 ml/g/min with 0.05–0.1 $\mu\text{g/g}$ body weight dose. This result demonstrates that maximal vasodilation was achieved with 0.05 $\mu\text{g/g}$ body weight of Regadenoson.

In this study we also demonstrated the ability of the sequence to quantify regional perfusions. Though there were no statistically significant differences in the rest and stress perfusion values between the different sectors, the perfusion in the anterior wall after vasodilation was slightly increased as compared to the other sectors. The high value in the anterior wall after vasodilation occurs because this area is prone to artifacts related to the nearby ascending and descending aorta, which has very high pulsatile flow after administration of Regadenoson.

Using the dual-contrast first-pass MRI sequence we detected reduced MPR in DIO mice fed a HFD for 24 weeks. A recent study looked at the long-term effects of a HFD in C57Bl/6 mice and showed that these mice exhibit several hallmarks of diabetic cardiomyopathy. In this study they showed that DIO mice fed a HFD for 8 months were overweight, hyperglycemic, insulin resistant, hypercholesterolemic, and hyperinsulinemic, and exhibited cardiomyocyte hypertrophy, cardiac fibrosis and a reduction in cardiac contractile reserve. In addition, these conditions worsened after 16 months of diet (22). Another recent study showed that DIO mice fed a HFD for 5 months have subtly abnormal cardiac mechanics (37). In the present study, we have shown that DIO mice fed a HFD for 24 weeks have reduced MPR, and this is consistent with prior data showing that human diabetic patients have reduced coronary flow reserve, even without obstructive coronary atherosclerosis (3). We also found that the DIO mice were severely overweight as compared to the wild type mice (55 ± 4 g vs. 31 ± 1 g, $p < 0.05$) and this is in agreement with prior studies (22, 37). As the present study focused on technique development and evaluation, we did not investigate the mechanisms underlying reduced perfusion reserve in the DIO mice.

First-pass contrast-enhanced MRI in mice has only recently been investigated (14–18). Coolen *et al.* (15) used a segmented acquisition with parallel imaging to perform first-pass MRI in mice with a temporal resolution of about 300–400 ms. They used this method to measure perfusion semi-quantitatively in mice at rest and post – myocardial infarction (MI) and showed that they could detect regional differences in the myocardium post-MI. However, they did not perform absolute quantification. Makowski *et al.* (17) used 10-fold k-t undersampling with a k-t principle component analysis image reconstruction technique to perform first-pass MRI in mice at rest and post-MI. They measured perfusion both semi-quantitatively and using Fermi-function deconvolution and reported MBF of 7.3 ± 1.5 ml/g/min in mice at rest, 7.1 ± 1.5 ml/g/min in the post-infarct remote myocardium and a reduced perfusion of 1.2 ± 0.8 ml/g/min in the infarcted segments of the myocardium. Our resting MBF value of approximately 5.7 ml/g/min is approximately 25% lower than Makowski's value. This difference could be due to the slightly lower isoflurane level used in

our study (1.25% in our study vs. 1.4% in Makowski *et al.*), as isoflurane is known to be a potent vasodilator and has specifically been shown to increase MBF in the mouse heart (10). In addition, Makowski *et al.* did not use a dual-bolus or dual-contrast technique, but rather assessed both the TF and AIF from a single slice acquired at a saturation delay of 100ms. Using our methods for computing MBF, we demonstrated that MBF will be overestimated using a single slice for the AIF and TF with a 100 ms delay. Jogiya *et al.* (16) used essentially identical methods as Makowski *et al.* to measure perfusion semi-quantitatively and used Fermi function deconvolution in mice at rest and after vasodilation with dipyridamole. They reported a resting MBF of 4.1 ± 0.5 ml/g/min that increased to 9.6 ± 2.5 ml/g/min after hyperemia, yielding a perfusion reserve of 2.4 ± 0.54 . Our rest and stress perfusion estimates are somewhat higher than the values reported in that study. Nierop *et al.* (18) used a dual-bolus approach to measure resting MBF in a cohort of 9 C57Bl6 mice, where the AIF was measured using a separate acquisition with a low dose of Gd-DTPA. They reported a mean MBF of 7.3 ± 0.9 ml/g/min at rest and demonstrated good repeatability of the method for measuring resting MBF in mice. Our measurement of resting MBF is lower than that reported in their study and this is likely due to the higher isoflurane level used in their study (1.5–2% vs. 1.25 % in our study).

Prior studies have measured MBF quantitatively in mice using other techniques such as arterial spin labeling (ASL) (9–13) and microspheres (38–40). Previously published studies using other techniques reported mean resting MBF of 4.3–7 ml/g/min and stress MBF of 8.2–14 ml/g/min in mice. Our perfusion estimates are centered within the ranges of these published values. The large variation in stress perfusion values is because of the different vasodilators and different doses used in the various studies. Our data is in good agreement with prior ASL data acquired in mice at rest (13, 36) and after administration of ATL313 (36). Since ASL requires longer acquisition times and perfusion estimation using microspheres is a terminal experiment in mice, first-pass MRI has significant advantages compared to both of these methods. Because first-pass MRI is performed so rapidly, it can easily be integrated into a comprehensive cardiac MRI exam for mice that assesses left-ventricular anatomy, function (41), strain (42, 43), and late gadolinium enhancement (5).

A limitation of this study is that no additional independent perfusion measurements were performed to validate the dual-contrast first-pass MRI technique. However, our perfusion estimates are within the ranges of previously published values. Another limitation is that our current imaging protocol only allows for perfusion measurement in a single slice. Increasing slice coverage is an important challenge for future technical development, which may be facilitated by using short echo-planar (44) or spiral trajectories (45). Image resolution may further be improved by using other k-space trajectories like radial or spiral, and by using higher acceleration rates possibly with phased-array coils and combining compressed sensing with parallel imaging (46). A further limitation of this study is that the dose-response studies were not all performed in the same set of mice and a separate set of mice was used for perfusion estimation using ATL313. In this manuscript, we have not studied perfusion in infarcted mice, however other groups have used first-pass MRI to image perfusion in post-infarct mice (14, 17). The heart rate in wild type C57Bl6 mice on day 1 post-infarct is typically around 550–600 bpm (13), which is similar to the heart rate we

observed after vasodilation in non-infarcted mice (563 ± 3 bpm). Given that the acquisition time for our dual-contrast sequence is 80 ms (using the sequence parameters stated in this study), we would not expect major problems in measuring perfusion in an acute infarct mouse model using the CS-accelerated dual-contrast sequence. However, in chronic infarcts after wall thinning occurs, the image resolution may be insufficient to accurately measure perfusion in the infarct scar.

Conclusions

In conclusion, k_y -t undersampled dual-contrast first-pass MRI with BLOSM reconstruction provides the spatial resolution, temporal resolution, and motion compensation suitable for quantifying myocardial perfusion in free-breathing mice using a single injection of a contrast agent. The dual-contrast approach uses separate slices with short and long saturation delays, and provides accurate measurements of MBF. With these methods, the scan time is sufficiently short so as to allow first-pass imaging at either rest or stress with vasodilation, providing for the assessment of MPR. Furthermore, we measured reduced MPR in DIO mice, which establishes an additional important abnormal parameter in this emerging mouse model of human diabetic cardiomyopathy. These techniques applied in gene-modified mice may be used to study mechanisms that underlie abnormal perfusion in cardiovascular diseases such as obesity and diabetes.

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Figure 1.

Diagram for the CS-accelerated dual-contrast first-pass MRI pulse sequence. A saturation pulse is applied after detection of the R wave and thereafter two slices are acquired in each cardiac cycle: 1) the AIF slice at acceleration rate 6 and at a saturation delay of 15 ms, with centric k-space ordering; 2) the TF slice at acceleration rate 4 and at a saturation delay of about 57 ms, with linear k-space ordering. The k_y - t sampling patterns for the two slices are also shown.

Figure 2.

Example images obtained from a mouse at rest. Arterial input function (AIF) images obtained using a short saturation delay (A–F) and tissue function (TF) images obtained using a longer saturation delay (G–L) show the arrival of contrast agent in the right ventricle (A, D, G, J), left-ventricular blood pool (B, E, H, K) and the myocardium (C, F, I, L). **(A–C):** Rate 6 undersampled AIF images reconstructed using a 2D- Fast Fourier Transform (FFT). **(D–F):** BLOSM-reconstructed AIF images. **(G–I):** Rate 4 undersampled TF images reconstructed using a 2D-FFT. **(J–L):** BLOSM-reconstructed TF images.

Figure 3.

(A): Example arterial input function (AIF) and tissue function (TF) data and fits obtained from a mouse at rest. **(B):** Example TF data and fits obtained from mice at rest (grey) and after vasodilation (black).

Figure 4.

Perfusion results obtained in wild type mice at rest and after vasodilation with ATL313 and Regadenoson. Perfusion was significantly increased with ATL313 and Regadenoson (* $p < 0.05$ vs. Rest).

Figure 5.

Dose-response curve obtained from wild-type mice at different doses of Regadenoson. MBF increased with increasing doses of Regadenoson, until a plateau was reached above 0.05 µg/g (*p<0.05 vs. rest, #p<0.05 vs. 0.01 µg/g).

Figure 6.

Example left-ventricular blood pool data and fits obtained from a mouse at rest using a short (10 ms, circles and solid black line) and a long saturation delay (57 ms, triangles and grey dashed line). These curves demonstrate that the AIF is underestimated when using a long saturation delay.

Figure 7.

(A): Example regional tissue function curves obtained from a mouse at rest. **(B):** Example regional tissue function curves obtained from a mouse after vasodilation. The myocardium was divided in 6 sectors (named clockwise starting from the RV insertion point): anterior, anterolateral, inferolateral, inferior, inferoseptal and anteroseptal. These curves demonstrate the ability of the CS-accelerated dual-contrast sequence to assess regional perfusions.

Figure 8.

(A): Rest and stress MBF results obtained in wild type and HFD mice after 24 weeks of diet. Rest MBF was similar in wild type and HFD mice. Stress MBF was increased in wild type mice (* $p < 0.05$ vs. wild type rest perfusion) and was reduced in HFD mice as compared to wild type mice (\$ $p < 0.05$ vs. wild type stress perfusion). (B): MPR results obtained in wild type and HFD mice after 24 weeks diet. MPR was reduced in the HFD mice (* $p < 0.05$ vs. wild type).

Table 1

Quantitative perfusion values (mean $\pm$ standard error) at rest and after Regadenoson administration when AIF is obtained using images with saturation delay 10 ms/57 ms

	Rest	Regadenoson (0.1 μg/g bodyweight)	Perfusion Reserve
Perfusion [ml/g/min] (AIF from images with Tsat = 10 ms)	5.7 $\pm$ 0.8	10.4 $\pm$ 0.3	2.0 $\pm$ 0.3
Perfusion [ml/g/min] (AIF from images with Tsat = 57 ms)	8.6 $\pm$ 1.4	18.4 $\pm$ 2	2.3 $\pm$ 0.4

Table 2

Regional perfusion values (mean $\pm$ standard error) obtained in the different myocardial sectors at rest and after Regadenoson administration

	Anterior	Antero-lateral	Infero-lateral	Inferior	Infero-septal	Antero-septal
Rest	5.9 $\pm$ 0.8	6.1 $\pm$ 1.8	5.5 $\pm$ 0.9	6.5 $\pm$ 0.8	6.8 $\pm$ 1.0	6.8 $\pm$ 0.6
Stress	16.1 $\pm$ 1.0*	11.3 $\pm$ 1.6*	10.8 $\pm$ 1.1*	11.8 $\pm$ 0.9*	14.3 $\pm$ 1.8*	13.8 $\pm$ 1.5*

* p < 0.05 vs. rest perfusion within the same sector