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RESEARCH ARTICLE

WILEY NMR IN BIOMEDICINE

Feasibility of coronary endothelial function assessment using arterial spin labeled CMR

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Coronary endothelial dysfunction (CED) is an independent predictor of cardiovascular disease, but its assessment has been limited to invasive coronary angiography. Myocardial perfusion imaging using arterial spin labeled (ASL) cardiac magnetic resonance (CMR) may be an effective non-invasive alternative for detection of CED.

Thirty-four patients were recruited: 10 healthy volunteers, 13 at high-risk for coronary artery disease (CAD), and 11 with established CAD. ASL-CMR was performed continuously in a single mid-short axis slice during rest, stress, and recovery. Stress was induced with sustained isometric handgrip exercise, an endothelial dependent stressor. Myocardial perfusion (MP) during rest, peak stress, and recovery were calculated and compared.

After excluding subjects unable to complete the protocol or who exhibited poor data quality, 6 healthy, 10 high-risk, and 7 CAD patients were included in the analysis. Average MP (ml/g/min) was 1.31 ± 1.23, 1.61 ± 1.12, and 1.40 ± 0.97 at rest, and 1.64 ± 1.49, 2.31 ± 1.61, and 2.84 ± 1.77 during stress, for the CAD, high-risk and healthy group, respectively. The average MP response (MP_{stress} – MP_{rest}, ml/g/min) was 0.32 ± 1.93, 0.69 ± 1.34, and 1.44 ± 1.46 for CAD, high-risk and healthy group, respectively. MP during handgrip stress was significantly lower for both the CAD (p = 0.0005) and high-risk groups (p = 0.05) compared to the healthy volunteers. In only the healthy subjects, MP was significantly higher in stress compared to rest (p = 0.0002).

Participants with CAD had significantly lower MP response compared to healthy volunteers, as detected by ASL-CMR. These findings support the feasibility of ASL-CMR for non-invasive assessment of CED.

KEYWORDS

applications, cardiovascular MR (CMR) methods, human study, ischemic heart disease, methods and engineering, myocardial perfusion, perfusion and permeability methods, perfusion spin labeling methods

Abbreviations used: MPI, Myocardial Perfusion Imaging; CED, Coronary Endothelial Dysfunction; CAD, Coronary Artery Disease; CBF, Coronary Blood Flow; CVD, Cardiovascular Disease; ASL, Arterial Spin Labeling; MP, Myocardial Perfusion; CMR, Cardiac Magnetic Resonance; MVC, Maximum Voluntary Contraction; IHG, Isometric Handgrip; PG, Plethysmograph Gating; FAIR, Flow Alternating Inversion Recovery; bSSFP, Balanced Steady State Free Precession; ROI, Region Of Interest; PN, Physiological Noise; tSNR, Temporal Snr; RLD, Restricted Likelihood Distance; HR, Heart Rate; PET, Positron Emission Tomography

Journal Subject Terms: Coronary circulation, Endothelium, magnetic resonance imaging, Coronary artery disease, Atherosclerosis, Translational studies, Clinical studies, Ischemia.

1 | BACKGROUND

The coronary endothelium plays a crucial role in vasomotor and blood flow regulation. In coronary endothelial dysfunction (**CED**), vasodilation is impaired and paradoxical vasoconstriction may occur with endothelial dependent stressors.[1] This may limit the increase in coronary blood flow **(CBF)** or cause a reduction in CBF with endothelial dependent stress. CED occurs in the initial stages of coronary atherosclerosis presumably in the presence of traditional cardiovascular risk factors[2] and is an independent predictor of cardiovascular disease (**CVD**),[3-7] the leading cause of mortality and morbidity in the developed world. These findings suggest the potential of CED detection to improve cardiovascular risk stratification or as a therapeutic target itself.

Despite the potential of CED assessment to improve CVD management, clinical application of coronary endothelial function testing has been limited due to a continued dependence of invasive techniques in the absence of proven non-invasive techniques. The gold standard for assessing CED remains coronary angiography,[6,8] which involves radiation, uses contrast agents, and has procedure-related complications. Peripheral endothelial function can be non-invasively studied with ultrasound, however, these vessels rarely develop plaque rupture and their function does not strongly correlate to that of the coronary arteries.[9-11] Current cardiac magnetic resonance (**CMR**) imaging methods to assess coronary endothelial function are effective but rely on high spatial resolution for visualization of the coronary arteries in cross-section.[12-14] Hence there remains a continued need to develop novel non-invasive techniques to directly study coronary endothelial function in at-risk asymptomatic populations.[13]

Arterial spin labeling (**ASL**)-CMR is a non-contrast technique for measuring myocardial perfusion (**MP**) and may be a viable alternative to assess CED that overcomes many of the limitations of current CMR techniques.[15-19] ASL-CMR requires lower spatial resolution than coronary MRI because signals are measured in the myocardium and not in the coronary tree. Additionally, MPI captures changes in the entire coronary arterial tree and, in essence, measures CBF to large regions of myocardium. Therefore, ASL-CMR may provide a more sensitive, non-invasive assessment of coronary endothelial function. We hypothesized that change in MP in response to endothelial dependent stress would be impaired in patients with coronary artery disease (**CAD**) and those at high-risk for CAD compared to healthy controls.

2 | METHODS

2.1 | Subject population

Thirty-four subjects with no contraindications to MRI underwent ASL-CMR imaging and were eligible for this study. The study subjects were divided among three groups: CAD (n = 11), high-risk for CAD (n = 13), and healthy (n = 10). CAD was defined as a[1] history consistent with angina or myocardial infarction based on clinical symptoms and non-invasive imaging and[2] a > 50% stenosis seen on prior coronary angiography in at least one major epicardial coronary artery. We defined high-risk patients as those with at-least 2 traditional CVD risk factors (hypertension, diabetes mellitus, active tobacco use, and hyperlipidemia) but[1] neither evidence of angina or myocardial infarction by clinical history and non-invasive imaging[2] nor angiographically-proven CAD (as defined above). Healthy participants were < 35 years of age and free of any traditional CVD risk factors or known CAD. This was established using a brief questionnaire documenting the absence of known heart disease, hypertension, smoking, diabetes and high cholesterol. The University of Southern California institutional review board approved the study protocol and informed consent was obtained from all subjects. Patients were also requested to refrain from caffeine for at-least 12 hours before the exam.

2.2 | Study protocol

Maximum voluntary contraction (**MVC**) was determined for each participant prior to beginning the imaging protocol and defined as the average of 3 maximum strength contractions of the isometric handgrip (**IHG**) device (Stoelting Co., Wood Dale, IL). All participants were scanned in the supine position. Images were acquired in a mid-short axis slice during stable diastasis throughout this study. ASL-CMR was used to measure MP continuously for 5–8 min of rest, 5 min of stress, and 10–12 min of recovery as shown in Figure 1. Stress was induced by sustained IHG exercise at 30%

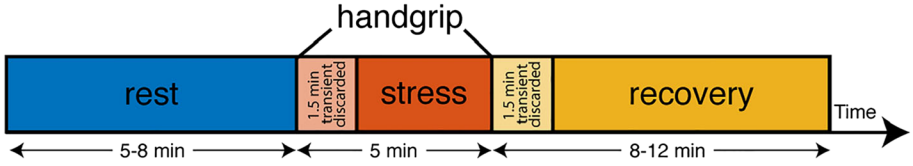

FIGURE 1 Scan protocol for coronary endothelial function testing using ASL-CMR. ASL-CMR was performed continuously for approximately 20–25 minutes: 5–8 min rest, 5 min handgrip stress, and 8–12 min rest. Data acquired during the periods marked rest, stress and recovery were each averaged to calculate MP for rest, stress, and recovery respectively. The initial 1.5 min of data during the stress and recovery phases reflect a transient a state and were therefore discarded

MVC. A research team member was present in the scan room with the subject to ensure compliance with IHG at the appropriate exertion level. This was done by directly reading force measurement on the IHG display or via verbal confirmation from the subjects who could read the IHG display. IHG device has previously been shown to be a repeatable endothelial dependent stressor.[12,14,20,21] Subjects were provided instructions to perform frequent 3–4 second breath-holds using a pre-recorded voice prompt from the scanner. Compliance with breathing instructions was confirmed by the scan operator monitoring the respiratory bellows signal in real-time. This reduced signal variation compared to free breathing and allowed for shorter breath-holds compared to 12–16 seconds for previous ASL-CMR protocols.[15,17,18,22]

Variation in the duration of rest and recovery period was primarily due to scan efficiency. Scan efficiency is defined as the number of control and label image pairs acquired per-unit of time (e.g. minute). A total for 54 MP measurements were collected for each subject over a duration of 20–25 min. For each subject; 18 MP measurements were collected during rest, 8–13 MP measurements were collected during 5 min of stress, and the remaining perfusion measurements were collected during recovery.

2.3 | MRI protocol

All experiments were performed on a 3 Tesla system (Signa Excite HDxt, GE Healthcare) using an eight-channel cardiac array and plethysmograph (**PG**) gating. ASL-CMR was performed using a double gated flow alternating inversion recovery (**FAIR**) with balanced steady state free precession (**bSSFP**) imaging.[17,19] Double gated FAIR sequence was used because it is less sensitive to expected heart rate variations during IHG stress.[17] Labeling was performed using a hyperbolic secant adiabatic inversion pulse with an inversion efficiency >85% in all subjects. Inversion efficiency was measured in-vivo by applying the inversion label immediately prior to image acquisition, as described by Do et al..[17] MR imaging parameters were: TE/TR = 1.5 ms/3.2 ms, matrix size: 96 x 96, spatial resolution: 1.88 mm–2.50 mm,FOV: 18 cm–24 cm, slice thickness: 1 cm, bandwidth per pixel: 651 Hz, GRAPPA *R* = 1.6 with 24 autocalibration signal (**ACS**) lines, image acquisition window: 192 ms, and flip angle = 50. The total duration for each experiment was ~45 min.

2.4 | Data analysis

All data analysis was performed in a blinded fashion. Data was processed using MATLAB (MathWorks Inc., South Natick, MA) as shown in Figure 2 . Similar to previous ASL-CMR studies all images were sinc interpolated by a factor of two to facilitate segmentation. Sinc interpolation was performed by zero-padding k-space before inverse Fourier transform. Semi-automated segmentation of the left ventricular myocardium was performed using offline motion correction with advanced normalization tools with previously published settings.[23]This generated masks for global and per-segment (six segment) analysis. The entire left ventricle myocardium from the slice was used as the region of interest (**ROI**) for global analysis, and the AHA six-segment model[24] of a mid-short-axis slice was used for per-segment analysis.[25]

MP was calculated using the interpolated signal difference (ΔM) from control and label T_1 curves as previously described by Poncelet et al.[26] and Do et al..[17] T1 curves for control and label images were fitted using established three parameter model for T1 mapping.[27] ΔM between control and label T1 curves was estimated at the average inversion time (TI_{avg}) of control and label images. MP was then calculated using the following equation:

$$\Delta M = \alpha M_0 f TI_{avg} e^{TI_{avg}/T1_{blood}},$$

derived from Buxton's general kinetic model,[28] where α is inversion efficiency, M_0 is equilibrium magnetization, f is myocardial perfusion, TI_{avg} is mean inversion time of control and label images, and T_{1blood} is the T_1 of arterial blood (assumed to be 1950 ms at 3 T based on Weingartner et al.[29]). Physiological noise (**PN**) (in ml/g/min) was calculated as the standard deviation of MP as described by Zun et al.[22] and is a measure of measurement variability. Temporal SNR (**tSNR**) was calculated as MP divided by PN and was used as a measure of data quality. MP values for rest, peak stress, and recovery period were calculated from the initial 5–8 min of rest data (18 pairs), 3.5 min of stress data (~6–8 pairs), and 10–12 min of recovery data (~20 pairs), respectively. The first 1.5 min of data during stress and recovery was not used in the calculation of MP because it was collected during a transient state. MP response was calculated as the difference in MP between stress and rest (i.e. MP stress – MP rest). Subjects were excluded from the study if: 1) the subject was unable to complete the study and verified this verbally after the scan, 2) there were technical difficulties with the imaging sequence, or if 3) the global tSNR was <1 during rest or stress, which indicates very low confidence (measurement variability is greater than the measured value). Individual pairs of MP measurements within a scan were rejected in case of a mis-trigger.

2.5 | Statistical analysis

Analysis was performed on the segmental MP measurements based on the AHA six-segment model for a mid-short axis slice. Data normality was examined using histograms. Mixed effect model was used to test the global intra- and inter-group interaction. Contrast tests were used for post

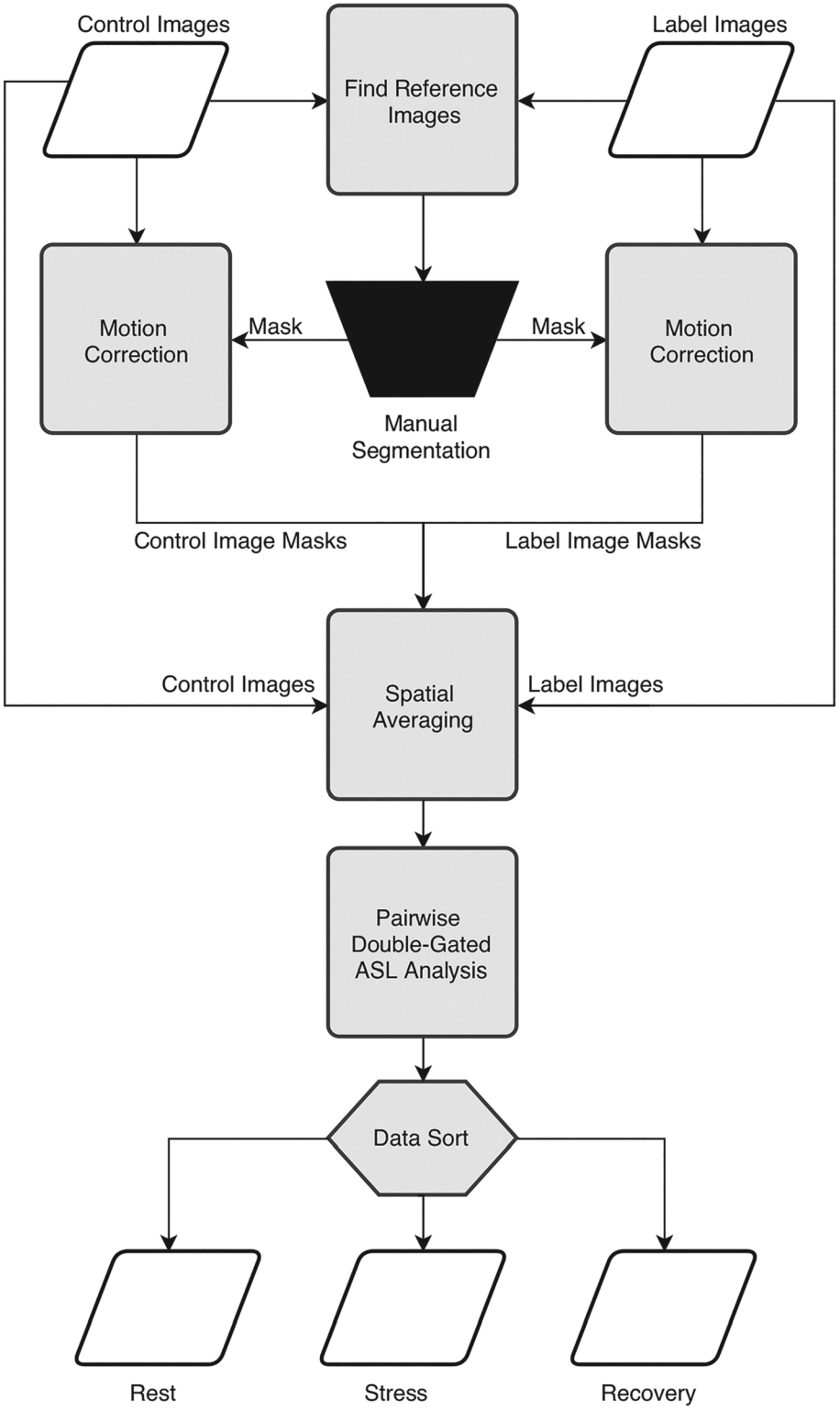

FIGURE 2 Flowchart for semi-automatic calculation of global and segmental MP for rest, stress and recovery data. Reference images are automatically selected from the control and label ASL-CMR images. Reference images are manually segmented to generate masks. Control and label images are then registered using motion correction software to their respective reference images and a reverse transformation is applied to the manually segmented masks to generate masks for each control and label image. These masks along with the control and label images are used for spatial averaging. These segmental and global values are used in double gated ASL-CMR analysis to generate MP measurements for each pair of control and label images. The data is then sorted into rest, stress, and recovery as shown in Figure 1

hoc comparisons, which included the tests for intra-group changes from rest to stress, inter-group difference for each measurement phase, and inter-group difference in changes from rest to stress. Bonferroni Step-down (**Holm**) correction was used to control the false discovery from multiple comparisons. Mixed model validity was examined using residual plots. Cook's D and restricted likelihood distance (**RLD**) were used to detect the potential outliers. If outliers detected, the sensitivity analysis was conducted by removing outliers. All data analyses were conducted using SAS 9.4.

3 | RESULTS

Thirty-four subjects were enrolled in the study. One healthy subject was unable to complete the MRI scan. Three CAD patients and one high-risk patient were excluded due to technical difficulties with the MRI pulse sequence, which resulted in poor image quality. Additionally, 6 subjects (3 healthy, 2 high-risk, and 1 CAD) were excluded due to inadequate tSNR. The remaining data from 23 subjects (6 healthy, 10 high-risk, and 7 CAD) were analyzed. This resulted in 42 CAD, 60 high-risk and 36 healthy myocardial segments available for analysis. Segmental tSNR was comparable for the CAD, high-risk, and healthy groups at 8.39 ± 8.1, 6.94 ± 5.1, and 8.57 ± 5.35, respectively. There was no substantial difference between the groups. These values were also comparable to previous studies.[15,22] Patient demographics and results are summarized in Table 1. Figure 5 shows representative image quality, MP, and tSNR in ASL-CMR.

The HR during rest, stress, and recovery is shown in Figure 3A with a statistically significant interaction ($p < 0.05$). There was a statistically significant increase in heart rate (**HR**) in all three patient groups following IHG stress. MP during rest, stress, and recovery is shown with group analysis in Figure 3B and for individual data in Figure 4 with a borderline statistically significant interaction ($p = 0.055$). The resting MP values reported in the study were comparable to previous ASL-CMR[15,22,30] and PET studies.[31] After correcting for multiple comparisons, the inter-group comparison showed no differences in MP between rest and recovery. Stress MP was significantly lower in both CAD ($p = 0.0005$) and high-risk groups (p = 0.05) compared to healthy volunteers. MP was significantly higher during stress compared to rest ($p = 0.0002$) for healthy controls but not for CAD or high-risk groups. Trends showed stronger MP response (stress-rest) in healthy (1.44 ± 1.46) and high-risk group (0.69 ± 1.34) compared to CAD group (0.32 ± 1.93). The statistical significance diminished after multiple comparison correction ($p = 0.12$ and 0.22 respectively).

Histograms showed that data from each measurement phase followed a bell shape distribution, but with slightly heavy tails, which may indicate the presence of outliers. After removing outliers the results remained the same.

TABLE 1 Demographic and measured MP, MP response and HR. values are reported as mean ± standard deviation and are only for subjects whose data were included in the final analysis

	Healthy	High-risk	CAD
Subjects			
n	6	10	7
Age, yrs	24 ± 1.7	68.5 ± 7.3	61.4 ± 11.8
Sex			
Male	5	6	5
Female	1	4	2
Hypertension	-	10	7
Hyperlipidemia	-	10	7
Diabetes mellitus	-	3	1
CAD			
Single-vessel	-	-	5
Multi-vessel	-	-	2
ASL			
# of segments	36	60	42
MP rest (ml/g/min)	1.4 ± 0.97	1.61 ± 1.12	1.31 ± 1.23
MP stress (ml/g/min)	2.84 ± 1.77	2.31 ± 1.61	1.64 ± 1.49
MP recovery (ml/g/min)	1.53 ± 1.14	1.34 ± 1.29	1.13 ± 0.96
MP response (ml/g/min)	1.44 ± 1.46	0.69 ± 1.34	0.32 ± 1.93
HR rest	60.4 ± 13.4	65.3 ± 14.1	65.1 ± 13.6
HR stress	72.3 ± 14.2	75 ± 14.7	71.6 ± 14.1

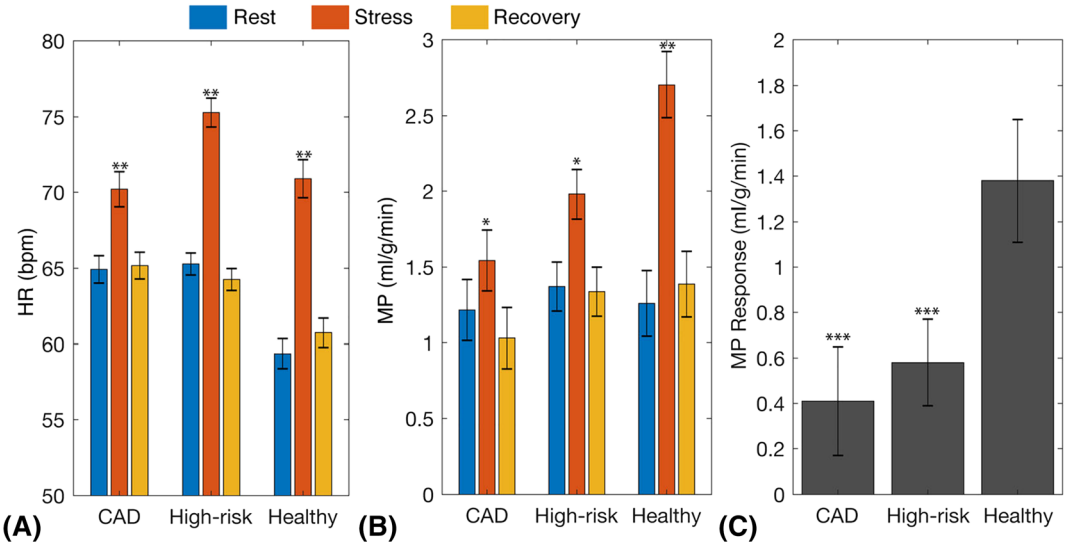

FIGURE 3 **A)** HR measurements during rest (blue), stress (orange) and recovery (yellow) during the ASL-CMR scans for CAD, high-risk and healthy groups. The error bars depict standard error. The HR difference between rest and stress was statistically significant (**$p < 0.05$) for all groups. **B)** MP measurements for the CAD, high-risk, and healthy groups. The difference between rest and stress MP was statistically significant for the healthy group (**p < 0.05). Stress MP was lower for the CAD and high-risk groups compared to the healthy group (*p < 0.05). Rest and recovery MP values were not statistically different in any of the three groups. **C)** MP response in the CAD, high-risk, and healthy groups. There was a statistically significant difference in MP response for healthy vs. CAD and healthy vs. high-risk with ***p < 0.05 before correction for multiple comparisons and not significant after correction. * denotes significance in comparison to healthy, ** denotes significance in relation to rest, and *** denotes significance only before correction when compared to healthy

4 | DISCUSSION

Coronary endothelial function, as determined by ASL-CMR, was significantly impaired in individuals with established CAD and those at increased risk for CAD compared to healthy participants. This study demonstrates the feasibility of using ASL-CMR determined MP to non-invasively measure of coronary endothelial function. MP during IHG exercise, an established endothelial-dependent stressor, was significantly lower for both the CAD and high-risk groups compared to the healthy volunteers. Additionally, stress MP was significantly higher than the rest MP only for the healthy volunteers.

The use of ASL-CMR to evaluate changes in MP to endothelial-independent vasodilation has previously been established and results presented here extend use for endothelial-dependent vasodilation.[18] Prior study has demonstrated the ability of CMR to assess coronary endothelial function using other methods. Phase contrast flow velocity imaging of the right coronary artery detected a significantly lower rise in coronary flow velocity in response to cold pressor test (**CPT**) for asymptomatic diabetic females compared to healthy females.[32] Hays and colleagues have extensively reported on the use of coronary MRA imaging to formally assess endothelial function.[12,13,33,34] In these studies, average CBF, based off of diameter and flow velocity changes in proximal coronary artery segments, was determined at rest, in response to IHG stress and in one study during recovery. They found that average CBF on cardiac MRI increased by >30%, decreased by 4%, and decreased by 13% in patients with no CAD, mild CAD, and significant CAD respectively. In patients where 2 separate coronary arteries were imaged, a greater degree of endothelial dysfunction was observed in the coronary artery with more severe stenosis compared to the contralateral, minimally diseased artery. Additionally, no difference in CBF was reported during rest and recovery periods.[12] Phase contrast imaging can also measure global coronary flow reserve in the coronary sinus.,[35,36] which in combination with IHG stress, may be viable for the assessment of CED.

While coronary endothelial function assessment with CMR can be determined with these other methods, some significant limitations exist. Imaging of the coronary arteries in CMR is limited by suboptimal spatial resolution, sensitivity to cardio-respiratory motion, and the ability to only image one or a handful of proximal coronary segments. CED may not affect all coronary arteries or microvasculature of a major coronary artery uniformly. ASL-CMR could potentially improve CED detection because it is not limited by spatial resolution requirements, is less sensitive to cardio-respiratory motion, and can potentially capture CED in the entire coronary tree by measuring changes in MP.

Positron emission tomography (**PET**) imaging has already validated the use of absolute MP to non-invasively determine coronary endothelial function and our findings are consistent with these prior studies.[37-39] PET requires injection of radiopharmaceuticals and involves radiation exposure, making it a poor choice for screening asymptomatic patients. Therefore, application of the MRI technique described here would be preferred in an asymptomatic population who requires further long-term cardiovascular risk stratification.

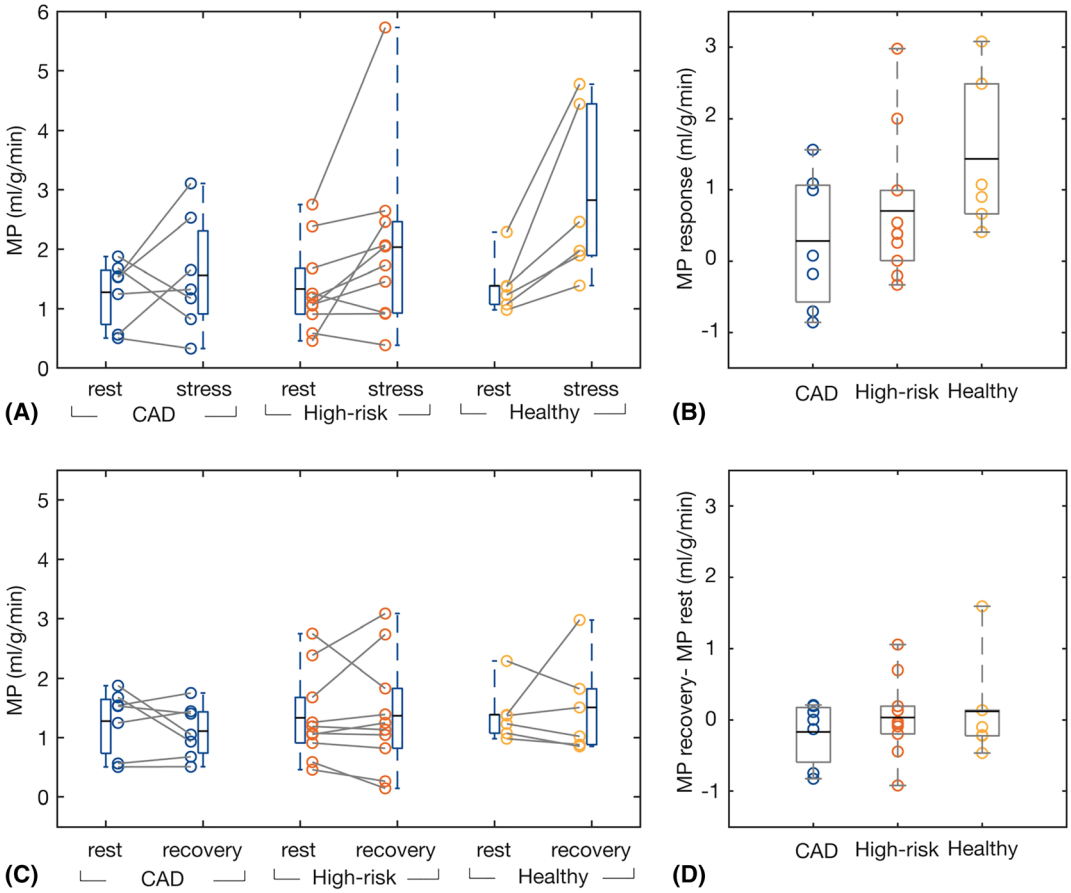

FIGURE 4 Individual subject MP data during rest, stress, and recovery for all three subject groups. (**a,B**) MP response was positive in all healthy subjects. In the high-risk and CAD groups, MP response was positive in some subjects and negative in some subjects, but on average the MP response was small. (**C,D**) we detected no significant difference between MP measured during rest and recovery, for all groups. This can serve as a measure of repeatability for the resting MP measurement

Despite the potential advantages of ASL-CMR compared to other currently available non-invasive techniques, adoption and widespread clinical use of this method will depend on not only its ability to reliably detect CED in asymptomatic individuals without evidence of CAD, but also whether such early identification results in therapies to improve coronary endothelial function and, ultimately, long-term cardiovascular outcomes. Prior studies involving PET-measured MP do suggest, however, that improvements in endothelium-related coronary artery function may have direct preventive effects on coronary atherosclerosis progression. Initiation of glucose-lowering treatment in diabetic patients significantly improved MP response during CPT testing, and the magnitude of MP improvement inversely correlated with progression of coronary artery calcium, an important subclinical CAD measure that strongly predicts development of future CVD events.[40,41] Similarly, decreases in body-mass index following gastric bypass surgery in morbidly obese individuals were associated with an improvement in MP during CPT. Finally, coronary endothelial function also improves following smoking cessation.[42] In a population of healthy, young individuals, the MP response to CPT significantly improved after just 1 month of smoking cessation such that no difference was seen between ex-smokers and non-smokers.[43]

Our study has limitations. ASL-CMR signal is primarily from MP, but also includes a contribution from blood volume which may be of independent value in the context of CED. Separation of the blood volume signal in ASL-CMR remains future work. ASL-CMR derived measures of coronary endothelial function were not directly compared to those obtained from coronary angiography. Since all study participants either had no known CHD or stable CHD, invasive testing would not have been appropriate. MP reserve assessment with pharmacologic vasodilation was not performed in this population and we cannot comment on whether co-existing microvascular disease may have also been present. Only a small number of subjects were recruited and findings presented need further validation in larger, well-characterized populations. The lack of a detected difference in rest vs. stress MP in CAD and high-risk patients could be due to the small sample size. Systematic evaluations of ASL-CMR on a larger cohort including patients with CAD are warranted for future study. We did not capture blood pressure at rest or during stress and MP-based assessment of coronary endothelial function may have been limited in the setting of severe hypertension. The healthy volunteers were not age-matched to the patient groups and, as a result, they were substantially younger. Considering the known age-related decline in coronary flow reserve that occurs, this age difference may have confounded the differences in coronary endothelial function observed here.[44-46] We wanted to ensure the healthy volunteers were free of cardiac risk factors and recruitment of such individuals with a similar average age to the patient groups

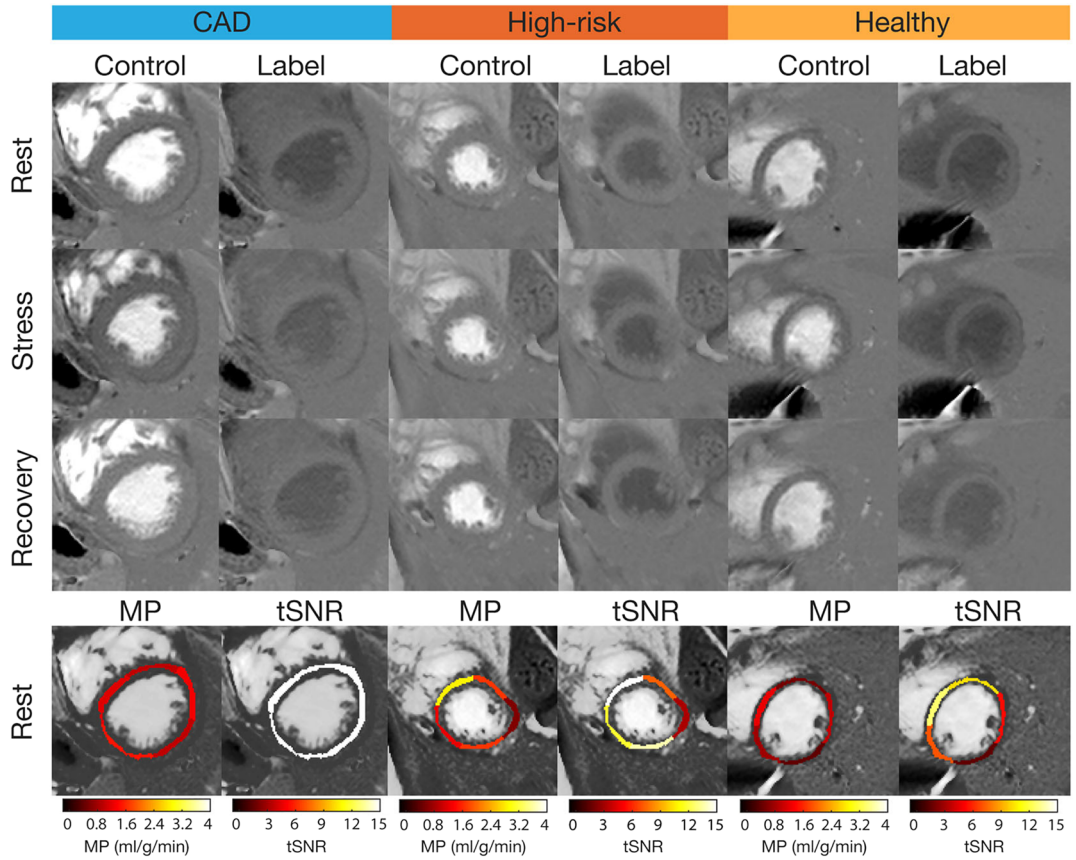

FIGURE 5 *Representative images, MP maps, and tSNR maps for (left) CAD, (middle) high-risk and (right) healthy subject groups.* ***(Top)*** *Control and label images acquired during rest, stress, and recovery. Image quality and SNR was high with good contrast between blood and myocardium.* ***(Bottom)*** *Resting MP maps and corresponding resting tSNR maps overlaid on control images. In these representative cases, resting MP values were comparable to previous ASL studies and segmental tSNR was high. Note that global tSNR was approximately 2x higher than segmental tSNR. Global tSNR < 1 was used as a rejection criterion in this study*

was difficult. Difficulties with the IHG device itself may have interfered with the ability to maintain 30% MVC as the device was bulky and participants needed to maintain an uncomfortable position in order to maintain a safe distance from the chest coil. Rate-pressure product could not be calculated because blood pressure data was not recorded. Therefore, only HR information was used to assess the hemodynamic response under stress. Finally, late gadolinium enhanced imaging was not performed, therefore we were unable to rule out the possible existence of infarct within the imaging slice for CAD patients.

In this study, we used GRAPPA acceleration (R = 1.6) with 24 auto-calibration lines (ACS), which we have found maximizes temporal SNR (tSNR) as shown in previous studies.[30] Acceleration beyond R = 1.6 leads to reduction in tSNR with our particular scanner setup and 8-channel cardiac coil. This is likely due to significant increase in thermal noise from g-factor losses. Further acceleration may be feasible with higher channel counts (16- or 32-channel coils) or other fast imaging sequences such as echo planar imaging (EPI).

In this study, the high failure rate was due to three reasons. 1) The first four subjects (3 CAD, 1 high-risk) were rejected due to poor image quality and un-resolved image artifacts. This was due to a sequence programming error. This implementation error was resolved completely and will not cause failures in future studies. 2) Six subjects (1 CAD, 2 high-risk, and 3 healthy) were rejected due to low tSNR. Previous ASL-CMR studies have reported global resting tSNR of 16 ± 8. In this study, the six subjects had resting tSNR <1. 3) One subject was rejected due to inability to complete the IHG stress.

5 | CONCLUSION

This study demonstrates the potential utility of ASL-CMR for non-invasive assessment of CED. The change in MP in response to IHG stress was progressively lower when going from healthy individuals to those at high-risk for CAD and, finally, to those with established CHD. Additional study with larger populations to further validate this technique for assessment of coronary endothelial function, including direct comparison with other available non-invasive CMR and PET methods, and to better establish reference range values with this method will be important.

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DISCLOSURES

The authors declare that they have no conflict of interests.

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