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**Cardiac Imaging**

# Noninvasive Visualization of Coronary Artery Endothelial Function in Healthy Subjects and in Patients With Coronary Artery Disease

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| **Objectives** | The goal was to test 2 hypotheses: first, that coronary endothelial function can be measured noninvasively and abnormal function detected using clinical 3.0-T magnetic resonance imaging (MRI); and second, that the extent of local coronary artery disease (CAD), in a given patient, is related to the degree of local abnormal coronary endothelial function. |
| **Background** | Abnormal endothelial function mediates the initiation and progression of atherosclerosis and predicts cardiovascular events. However, direct measures of coronary endothelial function have required invasive assessment. |
| **Methods** | The MRI was performed in 20 healthy adults and 17 patients with CAD. Cross-sectional coronary area and blood flow were quantified before and during isometric handgrip exercise, an endothelial-dependent stressor. In 10 severe, single-vessel CAD patients, paired endothelial function was measured in the artery with severe stenosis and the contralateral artery with minimal disease. |
| **Results** | In healthy adults, coronary arteries dilated and flow increased with stress. In CAD patients, coronary artery area and blood flow decreased with stress (both p ≤ 0.02). In the paired study, coronary artery area and blood flow failed to increase during exercise in the mildly diseased vessel, but both area (p = 0.01) and blood flow (p = 0.02) decreased significantly in the severely diseased, contralateral artery. |
| **Conclusions** | Endothelial-dependent coronary artery dilation and increased blood flow in healthy subjects, and their absence in CAD patients, can now be directly visualized and quantified noninvasively. Local coronary endothelial function differs between severely and mildly diseased arteries in a given CAD patient. This novel, safe method may offer new insights regarding the importance of local coronary endothelial function and improved risk stratification in patients at risk for and with known CAD.    (J Am Coll Cardiol 2010;56:1657–65) © 2010 by the American College of Cardiology Foundation |

Coronary vascular function plays a pivotal role in the development, progression, and clinical manifestations of coronary artery disease (CAD) (1). One defining characteristic of nondiseased vascular tissue is endothelial release of nitric oxide, which inhibits platelet aggregation, attenuates inflammation, decreases cellular proliferation, and induces local vascular smooth muscle vasodilation (2). The latter effect has been used to assess vascular function for >2 decades, by describing the direction and magnitude of changes in arterial diameter and flow velocity in response to endothelial-dependent vasomotor interventions (1). In addition to serving as a measure of dysfunction, the abnormal coronary vasomotor response is also a marker for subclinical

disease, an independent predictor of adverse cardiovascular events, and a potential target for medical interventions (3–9). Despite the importance of this measure of dynamic vasomotor function, much of our interpretation of its role is based on studies in peripheral vessels, whose function correlates only modestly with that of the coronary arteries (10–12). Although the noninvasive assessment of brachial

**Abbreviations
and Acronyms**

**CAD** = coronary artery
disease

**LCA** = left coronary artery

**MRI** = magnetic resonance
imaging

**RCA** = right coronary
artery

**RPP** = rate-pressure
product

artery endothelial function has been used as a surrogate measure of coronary endothelial function, the brachial arteries rarely develop severe atherosclerosis or plaque rupture. Moreover, endothelial injury plays a causal role in the development and progression of local atherosclerosis, a regionally heterogeneous process in the coronary arteries.

Traditionally, direct measurement of coronary endothelial function required invasive coronary angiography with Doppler flow measures to quantify the vasodilatory and flow responses to endothelial-dependent stressors. This invasive requirement has limited the extent of clinical and research investigations into coronary endothelial function, particularly those which would most benefit from repeated studies and/or studies in healthy subjects. Thus, a noninvasive, safe means to assess coronary endothelial function would be a potent clinical and research tool.

Magnetic resonance imaging (MRI) has been validated as a noninvasive means to assess coronary arterial cross-sectional area (13,14) and flow velocity (15,16). However, MRI has not been exploited to study coronary endothelial-dependent vasomotor responses in healthy and diseased states. Recently available, higher field, 3.0-T MRI scanners offer improved image quality and spatial and temporal resolution (17). Therefore, we combined 3.0-T coronary MRI with isometric handgrip exercise (18–20) to quantify coronary responses to an endothelial-dependent stressor and to test the hypothesis that coronary vasoreactivity can be measured and abnormal function detected noninvasively. Finally, given the central role that endothelial injury plays in

the local progression of coronary disease, we sought to test the hypothesis that local coronary endothelial function varies in a given patient and is more abnormal in coronary arteries with a significant stenosis than in arteries with mild stenotic disease.

## Methods

**Patients.** Subjects were outpatients with no contraindications to MRI. Healthy subjects were those <50 years of age without a history of CAD and traditional CAD risk factors, and also those >50 years of age with an Agatston coronary artery calcium score (21) of <10. Male sex and age were not counted when summarizing the risk factors in healthy subjects (Table 1). The CAD subjects all had CAD (>30% stenosis) on previous coronary X-ray angiography. For the substudy of 10 CAD patients who had both right coronary artery (RCA) and left coronary artery (LCA) imaged, the coronary X-ray angiogram was performed within 6 months of the MRI examination. The protocol was approved by the institutional review board at Johns Hopkins University, and all participants provided written informed consent.

**Study protocol.** The MRI was performed in the morning in the fasting state and before administration of prescribed vasoactive medications. Images were taken perpendicular to a proximal, linear segment of the coronary artery best identified on scout images (Fig. 1A). To ensure slice orientation perpendicular to the coronary artery, double oblique scout scanning was performed, as previously reported (22). To avoid partial volume effects related to the anisotropic spatial resolution, the imaging plane for endothelial function measurements was localized in a proximal or mid-arterial segment that was straight over a distance of 2 cm. To minimize bias of the analysis induced by stress, all

| Table 1 | Characteristics of the Subjects | | |
|---|---|---|---|
| | **Healthy Subjects (n = 20)** | **CAD Patients (n = 17)** | **p Value** |
| Age, yrs | 40.2 ± 13.7 | 55.5 ± 6.8 | <0.001 |
| Range | 24–67 | 47–67 | |
| Male | 12 (60) | 8 (47) | 0.45 |
| Left ventricular ejection fraction | N/A | 59 ± 11.2 | |
| Coronary artery imaged | | | |
| RCA alone | 19 (95) | 4 (23) | 0.25 |
| LCA alone | 1 (5) | 3 (18) | 0.25 |
| Both RCA and LCA | 0 | 10 (59) | <0.001 |
| CAD risk factors* | 0 | 3.5 ± 1.3 | <0.0001 |
| Multivessel disease | 0 | 3 (18) | 0.08 |
| History of PCI or CABG | 0 | 7 (41) | 0.004 |
| History of MI | 0 | 2 (12) | 0.16 |
| ACE-inhibitor use | 0 | 11 (65) | <0.0001 |
| Beta-blocker use | 0 | 13 (76) | <0.0001 |
| HMG-CoA reductase inhibitor use | 0 | 16 (94) | <0.0001 |

Values are mean ± SD or n (%) unless otherwise indicated. *Coronary artery disease (CAD) risk factors excluding age and sex.

ACE = angiotensin-converting enzyme; CABG = coronary artery bypass graft surgery; HMG-CoA = 3-hydroxy-3-methyl-glutaryl-coenzyme-A; LCA = left coronary artery; MI = myocardial infarction; N/A = not available; PCI = percutaneous coronary intervention; RCA = right coronary artery.
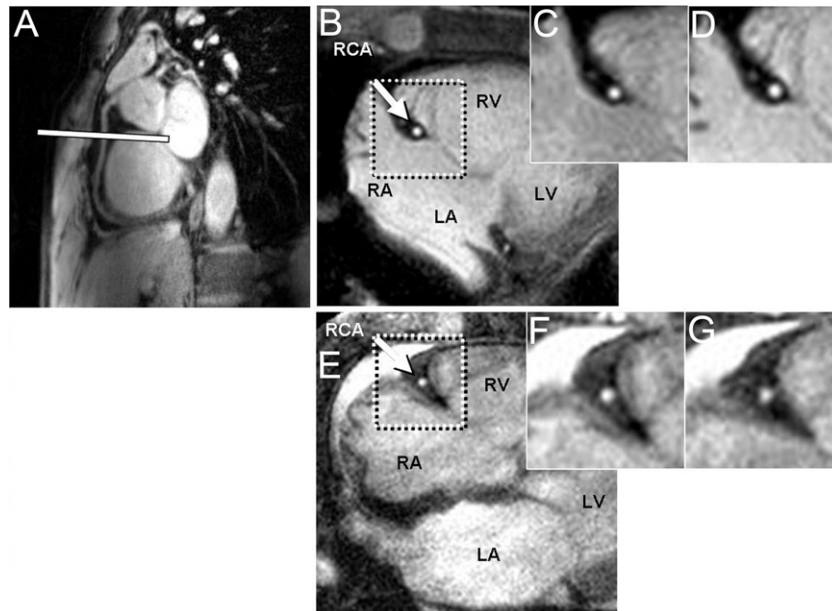
**Figure 1    Typical Anatomical Coronary Images Using MRI at Rest and With Isometric Handgrip Stress**

**(A)** In this magnetic resonance imaging (MRI) scan, a scout scan obtained parallel to the right coronary artery (RCA) in a healthy adult subject is shown together with the location for cross-sectional imaging **(white line)**. **(B)** A view from a healthy adult subject perpendicular to the RCA **(white arrow)** is shown. The **dotted rectangle** in **B** is magnified and shows the region that was selected for analysis at rest **(C)** and during stress **(D)**. **(E)** A similarly planned perpendicular RCA cross-sectional image obtained in a coronary artery disease (CAD) patient. Magnification of the corresponding RCA cross section at rest **(F)** and during stress **(G)**. LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle.

quantifications were performed during the period of least cardiac motion.

Baseline imaging at rest for cross-sectional coronary artery area measurements (23) (Fig. 1B) was followed by coronary flow velocity-encoded MRI (24) (Fig. 2A). Alternating anatomical and velocity-encoded images were collected at baseline, during 4.5 min of continuous isometric handgrip, and for ≤12 min during recovery. Each subject performed sustained isometric handgrip exercise using an MRI-compatible dynamometer (Stoelting, Wood Dale, Illinois) at 30% of his or her maximum grip strength (20) under direct supervision. After 10 min of recovery, a subset of healthy volunteers and CAD patients additionally received 0.4 mg of sublingual nitroglycerin, and imaging was repeated (25). Heart rate and blood pressure were measured throughout using a noninvasive and MRI-compatible electrocardiogram and calf blood pressure monitor (Invivo, Precess, Orlando, Florida). The rate-pressure product (RPP) was calculated as systolic blood pressure × heart rate.

In a substudy of 10 CAD patients with severe single-vessel disease, 2 arteries per patient were imaged in a proximal segment: 1 artery (LCA or RCA) with a significant stenosis (>60%) and the contralateral artery with noncritical disease (<30%) by X-ray angiography. In the arteries with severe stenosis, the cross-sectional imaging plane was located in an unaffected region at least 2 cm proximal to the location of the stenosis.

Ten healthy adult subjects re-entered the MRI scanner 20 to 60 min after the first examination and had the entire MRI examination repeated to determine reproducibility of the MRI handgrip protocol results.
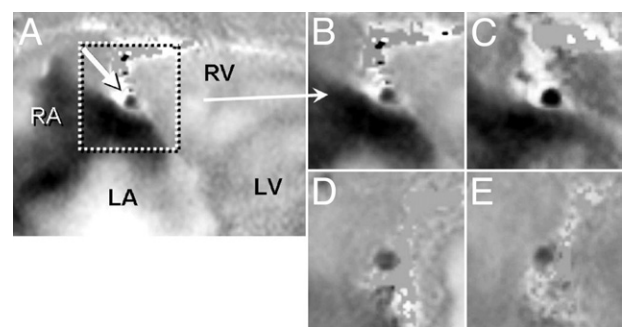


**Figure 2    Flow Velocity-Encoded MRI Phase Images**

**(A)** In this MRI scan, the **short white arrow** shows a cross section of the RCA that was selected for analysis for coronary flow velocity measurements in a healthy volunteer. The signal intensity is proportional to flow velocity, with a black signal indicating high velocity down through the imaging plane. In the magnified view of the RCA at baseline **(B)** and during isometric handgrip stress **(C)**, the decrease in luminal coronary signal intensity (increased blackness) illustrates an increase in through-plane coronary flow velocity. The cross section of the RCA in a coronary artery disease patient at rest **(D)** and during handgrip stress **(E)** indicates no increase in through-plane coronary flow velocity during stress. Abbreviations as in Figure 1.

**MRI.** A commercial human 3.0-T MRI scanner (Achieva, Philips, Best, the Netherlands) with a 6-element cardiac coil for signal reception was used. Cross-sectional anatomical (23) and flow velocity encoded spiral MRI (24) were performed using single breath-hold cine sequences (25). The temporal/spatial resolution for the anatomical images was 15 ms/0.89 × 0.89 × 8.0 mm$^3$ and 34 ms/0.8 × 0.8 × 8 mm$^3$ for the flow velocity images collected with a velocity encoding of 35 cm/s. The total duration of the MRI was ~40 min.

**Image analysis.** Images were analyzed for cross-sectional area changes using a semiautomated software tool (Cine version 3.15.17, General Electric, Milwaukee, Wisconsin). A circular region of interest was manually traced around the coronary artery in diastole when the least coronary motion occurred. The computer algorithm then measured the cross-sectional coronary area using an automated full width half maximum algorithm.

For flow measurements, images were analyzed using commercial software (FLOW version 3.0, Medis, the Netherlands). Peak diastolic coronary flow velocity was used for the velocity measurement because adverse effects of motion blurring and through-plane motion are minimized in mid-diastole, and because this maximizes the dynamic range of flow measurements. To account for baseline phase offset and that induced by through-plane motion, the coronary blood flow velocity was computed relative to the velocity in a myocardial region of interest adjacent to the coronary artery of interest (16). If aliasing occurred, phase unwrapping was performed by the FLOW software. Coronary flow velocity reserve was also computed (peak diastolic coronary flow velocity during stress divided by that at baseline). To calculate coronary artery blood flow (ml/min), the following equation was used: coronary artery cross-sectional area × coronary artery peak diastolic velocity × 0.3 (26). Images were analyzed by 2 readers (G.H., A.H.) blinded to both subject group (healthy vs. CAD) and state (rest vs. stress).

**Statistical analysis.** Data are expressed as mean value ± 1 SD. Proportions were compared using Fisher exact tests. Paired Student $t$ tests were used to compare baseline and stress coronary artery cross-sectional area, diastolic coronary flow velocity, and blood flow measurements. Paired $t$ tests were also used for the contralateral comparisons. Student unpaired $t$ tests were used to compare the changes from rest to stress in coronary cross-sectional area, peak diastolic coronary flow velocity, and blood flow measurements between the healthy subjects and the CAD subjects. No adjustments were made for multiple comparisons. The STATA version 9.2 software (StataCorp, College Station, Texas) was used for statistical analyses. The Bland-Altman method was used to assess interobserver, intraobserver, and intrasubject agreement for area, peak diastolic velocity, and coronary blood flow measurements, with p values derived from Pitman's test of differences. Statistical significance was defined as a 2-tailed p value <0.05.
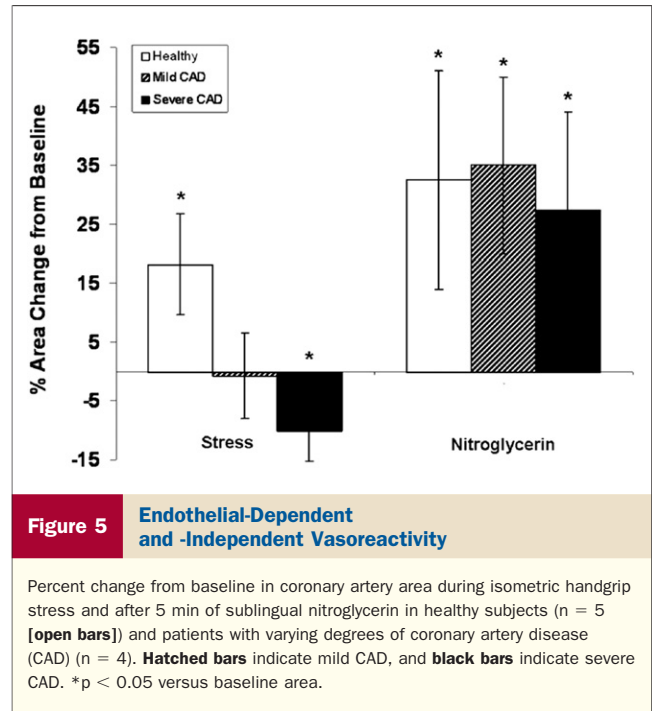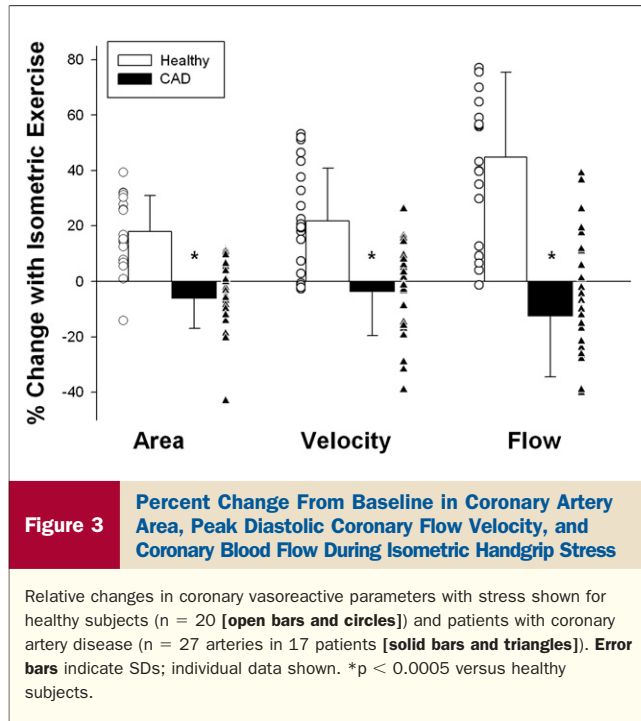
## Results

The baseline characteristics of the study population are presented in Table 1. All subjects completed the study without complication. One CAD patient was excluded from analysis because of motion artifacts. For the primary study, 27 coronary arteries were imaged in 17 CAD patients. Eleven of the 27 arteries in CAD patients had a significant (>60%) focal stenosis on prior X-ray angiography, whereas the remainder (16 of 27) had mild luminal disease (<30% stenosis) in the artery imaged. In the substudy assessing the relationship between the severity of stenotic disease and the degree of coronary endothelial dysfunction, both the RCA and LCA were imaged in each of the 10 CAD patients. These patients had only single-vessel significant CAD on cardiac catheterization, with >60% stenosis in either the RCA or LCA and <30% stenosis in the contralateral vessel.

**Hemodynamic effect of isometric handgrip stress.** Isometric handgrip exercise caused a modest but significant hemodynamic effect in both groups. In healthy subjects, there was a 12.5% increase in mean systolic blood pressure (p < 0.0001) and a 15.9% increase in mean heart rate with stress (p < 0.0001); whereas in CAD patients, the increases were 12.5% and 12.6%, respectively (both p < 0.0001 vs. baseline). The RPP increased by 27% with stress (p < 0.0001) in healthy subjects to 10,836 ± 2,316 mm Hg · beats/min, and it increased by 26% (p < 0.0001) in CAD patients to 11,971 ± 2,274 mm Hg · beats/min. The RPP during stress and the percent increase in RPP from baseline did not significantly differ between CAD patients and healthy subjects.

**Coronary vasodilation.** Representative anatomical coronary images are shown in Figure 1. In the healthy group, coronary arteries dilated significantly with stress (baseline cross-sectional area 12.8 ± 3.4 mm$^2$ vs. stress 15.1 ± 4.4 mm$^2$, p < 0.0001) and returned to baseline within 3 min of recovery. In the CAD group, coronary artery area decreased slightly with stress (baseline area 14.9 ± 5.3 mm$^2$ vs. stress 14.0 ± 5.0 mm$^2$, p = 0.02) and returned to baseline by 3 min of recovery. The relative stress-induced area change was significantly larger in healthy subjects (18.0 ± 13.0%) when compared with CAD (−6.0 ± 11.4%, p < 0.0001) (Fig. 3).

In the subset of CAD patients with 2 coronary arteries imaged, isometric handgrip stress decreased cross-sectional area in arteries with severe CAD (baseline 15.2 ± 4.1 mm$^2$ vs. stress 13.4 ± 3.7 mm$^2$, p = 0.01) but did not change area in the contralateral arteries with minimal disease (baseline area 12.4 ± 3.6 mm$^2$ vs. stress 12.2 ± 3.8 mm$^2$, p = 0.43). The relative area change with stress was significantly greater in the severely stenosed arteries (−11.5 ± 12%) than in arteries with mild stenosis (−1.7 ± 7.3%, p = 0.01 mild vs. severe) (Fig. 4).

In healthy volunteers (n = 5) and CAD patients (n = 4) who also received sublingual nitroglycerin, nitroglycerin increased coronary artery area in healthy and diseased

**Figure 3**   **Percent Change From Baseline in Coronary Artery Area, Peak Diastolic Coronary Flow Velocity, and Coronary Blood Flow During Isometric Handgrip Stress**

Relative changes in coronary vasoreactive parameters with stress shown for healthy subjects (n = 20 [open bars and circles]) and patients with coronary artery disease (n = 27 arteries in 17 patients [solid bars and triangles]). **Error bars** indicate SDs; individual data shown. *p < 0.0005 versus healthy subjects.



**Figure 5**   **Endothelial-Dependent and -Independent Vasoreactivity**

Percent change from baseline in coronary artery area during isometric handgrip stress and after 5 min of sublingual nitroglycerin in healthy subjects (n = 5 [open bars]) and patients with varying degrees of coronary artery disease (CAD) (n = 4). **Hatched bars** indicate mild CAD, and **black bars** indicate severe CAD. *p < 0.05 versus baseline area.

segments without a statistically significant difference in percent area change between the 2 groups (Fig. 5).

**Coronary flow velocity and blood flow measures.** Representative flow images are displayed in Figure 2. Peak diastolic coronary flow velocity increased in healthy subjects with stress (p < 0.001) and returned to baseline by 3 min of recovery. In contrast, there was no increase for CAD



**Figure 4**   **Percent Change From Baseline in Coronary Vasoreactive Parameters**

Percent change in coronary artery area, peak diastolic coronary flow velocity, and blood flow during isometric handgrip stress for healthy subjects (n = 20 [open bars]) and patients with single-vessel coronary artery disease (CAD) (n = 10). **Brackets** show comparisons between arteries with mild CAD (**hatched bars**) versus severe CAD (**solid bars**) within the same patients. *p < 0.005, †p < 0.001 versus healthy subjects.

subjects. The relative exercise-induced change in peak diastolic coronary flow velocity was greater in healthy subjects (21.8 ± 19%) than in CAD subjects (−3.5 ± 16%, p = 0.0003) (Fig. 3).

In healthy subjects, coronary blood flow increased significantly with isometric handgrip (79.6 ± 30.7 ml/min vs. 115.3 ± 53.1 ml/min, p < 0.0001), whereas blood flow decreased with stress in CAD (113.2 ± 49.2 ml/min vs. 99.1 ± 32.7 ml/min, p = 0.01). Coronary blood flow changes with stress in absolute terms (35.7 ± 30.5 ml/min vs. −14.1 ± 27.0 ml/min, p < 0.00001) and in relative terms (44.8 ± 30.6% vs. −12.5 ± 22%, p < 0.00001) (Fig. 3) were greater in healthy subjects than in CAD subjects, respectively. Among CAD patients with both coronary arteries imaged, coronary blood flow was unchanged during stress in mildly diseased arteries but was significantly reduced with stress in severely diseased ones (p = 0.02) (Fig. 4). However, there was no significant difference in change of peak diastolic flow velocity in response to handgrip stress between the 2 arteries of varying disease severity.

Mean coronary flow velocity reserve was significantly higher for the healthy subjects (1.24 ± 0.24) than for CAD subjects (1.01 ± 0.17, p < 0.0001).

**Time course of vasoreactivity during stress.** To determine whether coronary area or flow indexes change during the course of isometric handgrip exercise, these measures were obtained during both the early portion (at 30 s) and late portion (at 3 min and 30 s) of the 4.5-min exercise interval in a subgroup of 16 healthy volunteers and 8 CAD patients. The mean cross-sectional areas obtained during the early and later time periods did not differ in either the healthy group (14.3 ± 3.5 mm$^2$ vs. 14.6 ± 3.5 mm$^2$,
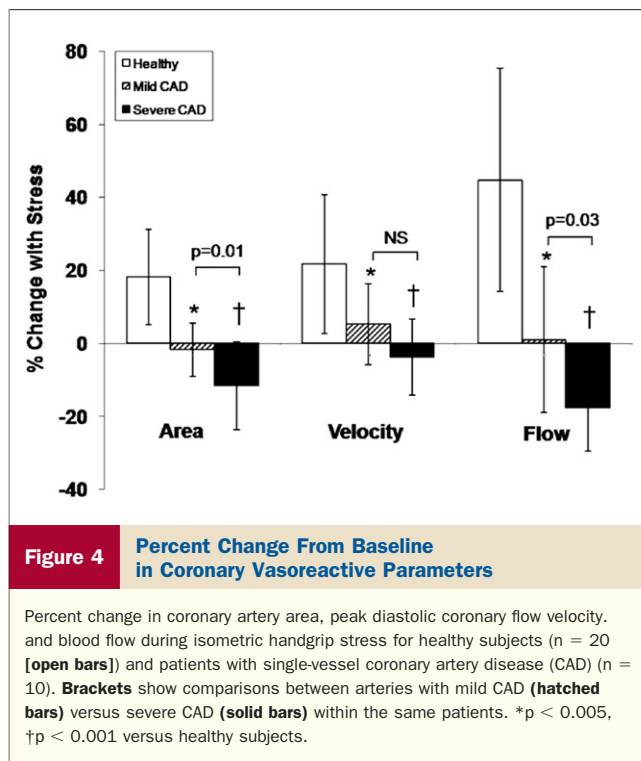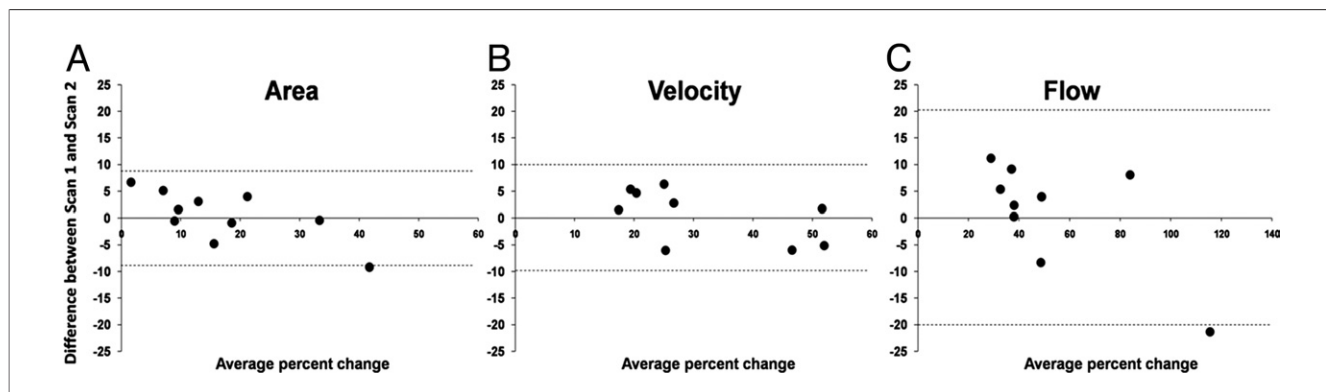
**Figure 6**   **Bland-Altman Plots Comparing Individual Subject Coronary Endothelial MRI Measurements in 2 Separate MRI Examinations**

**(A)** The percent area change between 2 separate magnetic resonance imaging (MRI) examinations is displayed (n = 10; p = 0.14). **(B)** The percent velocity change comparisons are displayed (n = 9; p = 0.24). **(C)** Percent absolute blood flow change between examinations is displayed (n = 9; p = 0.18). **Dashed lines** represent ± 2 SD, and the mean differences are shown. The p values are derived from Pitman's test of differences.

p = NS) or the CAD group (15.6 ± 1.3 mm$^2$ vs. 16.1 ± 1.2 mm$^2$, p = NS). There were also no significant differences in coronary flow between the early and late stress time points (nonblinded data) in both groups.

**Reproducibility.** Among the subjects who had the entire MRI protocol repeated, there were no significant differences in the exercise-induced changes in percentage area, peak diastolic velocity, or blood flow between the 2 studies. The Bland-Altman plots are presented in Figure 6. We also performed Bland-Altman analyses of intraobserver and interobserver variability of the area and velocity changes. The intraobserver results for area and velocity changes showed no significant differences (p = 0.14 and p = 0.24, respectively). Similarly, the interobserver variability for measurement of area and velocity changes did not show significant differences (p = 0.17 and p = 0.56, respectively).

## Discussion

3.0-T MRI was performed before, during, and after isometric handgrip exercise, an established endothelial-dependent stressor. Physiologic coronary artery vasodilation, increased coronary flow velocity, and absolute coronary blood flow were noninvasively observed in healthy subjects but not in CAD patients. Moreover, we detected differences in local endothelial function, with more severe impairment of endothelial function in arteries with more advanced CAD than in contralateral, mildly diseased vessels in single-vessel CAD patients. The ability to noninvasively characterize coronary endothelial function provides an opportunity to expand our understanding of the dynamic, pathophysiologic, and local role of coronary endothelial function in CAD, and may therefore contribute to the identification of high-risk lesions and the monitoring of factors promoting atherosclerotic progression at an early and pre-clinical stage.

From the seminal description of paradoxical coronary vasoconstriction in response to a vasorelaxant substance in CAD patients (1), the assessment of endothelial-dependent coronary vasoreactivity during cardiac catheterization has been widely studied to characterize endothelial function. Coronary endothelial function described using those methods provides important prognostic information for patients with CAD risk factors, and predicts future cardiovascular events (6–9,27,28). The findings that pharmacologic and lifestyle interventions known to reduce cardiovascular risk also improve coronary endothelial function (3–5,29) are consistent with the growing understanding that changes in endothelial function have important implications for future clinical manifestations of cardiovascular disease. Although the value of directly measuring coronary endothelial vasoreactivity is well established, the invasive nature of prior approaches limited the populations that could be studied and its use in clinical practice.

A large number of studies, therefore, used brachial artery measures to assess systemic endothelial function (30–32). Although those studies provide important insights, the correlation between peripheral and coronary endothelial function is modest (10,12), possibly related to differences in local vascular biology (11) and clinical events, as acute brachial arterial plaque rupture rarely occurs, in contrast to acute coronary syndromes. Thus, the noninvasive measurement of coronary endothelial function is arguably more relevant for defining factors related to local coronary artery atherosclerosis, responsible mechanisms, and the effect of interventions designed to improve clinical outcomes in CAD patients. Positron emission tomography techniques have been used to identify changes in segmental myocardial perfusion in response to endothelial-dependent stressors (33), and although they are noninvasive, such approaches do not directly visualize the changes in coronary artery area and coronary velocity or flow that define and characterize endothelial-dependent vasomotor responses.

Although MRI has been used to quantify endothelial-independent functional changes in both coronary blood flow (34) and diameter in response to nitroglycerin (25), our

study employed an endothelial-dependent stressor and combined both anatomic and flow measures in a single examination to obtain a dynamic, physiologic visualization and assessment of coronary artery endothelial function.

The cross-sectional area measures are local, whereas those of blood flow are more global, depending on both local vasoreactivity and distal microcirculatory runoff. Handgrip exercise increased area by ~18% in healthy subjects and decreased it by 6% in CAD patients. The vasoconstrictor response in CAD patients occurred even though many were taking medications, such as statins and angiotensin-converting enzyme inhibitors, known to improve endothelial function (3,9). Although vasoactive medications were held during the morning of the study, there likely were lingering effects still present at the time of the examinations. In addition, we observed an approximate 22% increase in peak coronary flow velocity in response to isometric handgrip stress in healthy subjects, in contrast to a 3.5% decline in CAD patients. Prior invasive coronary artery studies demonstrated similar increases in cross-sectional area and peak diastolic coronary flow velocity, although the endothelial-dependent stressors varied among studies (1,7,19,35). In a very early coronary MRI study of healthy subjects, a comparable increase in coronary flow velocity was reported (36), although our average baseline peak coronary flow velocity is closer to values obtained from Doppler guidewire studies (37,38), possibly related to higher spatial and temporal resolution with 3.0-T MRI. The ability to noninvasively measure endothelial-dependent changes in coronary area and flow velocity in a single examination, as described here for the first time, enables calculation of coronary flow, a more comprehensive measure of endothelial function with a large dynamic range for detecting differences between healthy and diseased states.

We observed a greater degree of endothelial dysfunction in the coronary artery with more severe stenotic disease than in the contralateral, minimally diseased artery exposed to identical systemic and genetic risk factors (Fig. 4). These observations suggest that coronary endothelial dysfunction is a regionally heterogeneous process, within a given patient, that may reflect local differences in the vascular microenvironment predisposing to the development and/or progression of atherosclerosis.

The finding that the administration of nitroglycerin to CAD patients dilated the same arteries that constricted by isometric handgrip exercise demonstrates that endothelial-independent mechanisms are intact, and that the mechanism responsible for the impaired handgrip-related response in CAD is abnormal endothelial function rather than a mechanical disturbance such as may occur with heavy coronary calcification or stenoses proximal or distal to the imaged segment. Although the detection of coronary vasodilation and flow in response to nitroglycerin and other endothelial-independent factors by magnetic resonance angiography has been previously reported (15,25,39), our study provides a novel noninvasive means to measure endothelial-dependent coronary vasoreactivity in humans with amplified differences between healthy and diseased vessels (Fig. 5).

Alternative interventions used to assess endothelial-dependent coronary artery vasoreactivity include cold pressor testing and intravenous or intracoronary acetylcholine (1,6,40). Isometric handgrip exercise is well suited to the MRI environment as it requires no vascular access and has been used safely in subjects with CAD (20). Furthermore, we observed that the reproducibility of the MR protocol had good intraobserver, interobserver, and intrasubject agreement. The reproducibility and fast changes in coronary area and flow (within 30 s) in response to handgrip should guide the design of future rapid, safe protocols using MRI assessment of endothelial-dependent vasoreactivity.

Computed tomography angiography is emerging as a robust, noninvasive, high-resolution tool for assessing coronary artery anatomy. However, its application to studies of coronary endothelial function is limited, as coronary blood flow velocity cannot be quantified. In addition, the radiation and contrast doses preclude stress studies in healthy and low-risk subjects or repeated studies in patients over time.

**Study limitations.** One limitation to this study is that we did not compare MRI-derived measures of coronary artery vasoreactivity with those obtained using invasive coronary angiography or Doppler guidewire. As many of the subjects were healthy, an invasive coronary test that was not clinically indicated could not be justified. Nevertheless, the fundamental MRI measures of coronary area (13,14) and blood flow velocity (15,16) were validated extensively before, and our results in terms of both direction and magnitude of the coronary responses corroborate those reported previously using invasive techniques (1,7,19,35). In addition, spatial resolution constraints and anisotropy of the voxel size currently limit the choice of the coronary segment that can be imaged. While we have primarily focused on proximal coronary segments that were nontortuous and were distant from luminal narrowings, more flexibility in the choice of the imaging plane will be required to account for the heterogeneity of local atherosclerotic disease progression. Therefore, technical developments toward volumetric image data acquisition with isotropic voxel size are likely to advance endothelial function studies. Lastly, another limitation is that most of the vessels studied in the healthy group were in the RCA. Although in this study we did not directly compare the vasoreactive responses between the left anterior descending artery and the RCA in healthy subjects, future studies can be designed using this technique to address this issue.

## Conclusions

The present study demonstrates that high-resolution MRI using commercial hardware and software, combined with isometric handgrip stress, provides a powerful new approach to the noninvasive assessment of endothelial-dependent

coronary vasoreactivity. This approach will permit, for the first time, the direct evaluation of coronary endothelial function in low-risk populations as well as repeated studies in patients over time. Our findings also demonstrate local differences in coronary endothelial function related to disease severity. Although measures of both peripheral and coronary endothelial function have been related to future events (7,41–43), these regional coronary observations raise doubts about the ability of a single measure of endothelial function in a peripheral vessel to accurately reflect the spectrum of endothelial function present in diseased coronary arteries in a given patient.

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**Key Words:** coronary disease ■ endothelium ■ magnetic resonance imaging.

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