Simultaneous Noninvasive Assessment of Systemic and Coronary Endothelial Function

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Background—Normal endothelial function is a measure of vascular health and dysfunction is a predictor of coronary events. Nitric oxide-mediated coronary artery endothelial function, as assessed by vasomotor reactivity during isometric handgrip exercise (IHE), was recently quantified noninvasively with magnetic resonance imaging (MRI). Because the internal mammary artery (IMA) is often visualized during coronary MRI, we propose the strategy of simultaneously assessing systemic and coronary endothelial function noninvasively by MRI during IHE.

Methods and Results—Changes in cross-sectional area and blood flow in the right coronary artery and the IMA in 25 patients with coronary artery disease and 26 healthy subjects during IHE were assessed using 3T MRI. In 8 healthy subjects, a nitric oxide synthase inhibitor was infused to evaluate the role of nitric oxide in the IMA-IHE response. Interobserver IMA-IHE reproducibility was good for cross-sectional area (R=0.91) and blood flow (R=0.91). In healthy subjects, cross-sectional area and blood flow of the IMA increased during IHE, and these responses were significantly attenuated by monomethyl-l-arginine (P<0.01 versus placebo). In patients with coronary artery disease, the right coronary artery did not dilate with IHE, and dilation of the IMA was less than that of the healthy subjects (P=0.01). The blood flow responses of both the right coronary artery and IMA to IHE were also significantly reduced in patients with coronary artery disease.

Conclusions—MRI-detected IMA responses to IHE primarily reflect nitric oxide-dependent endothelial function and are reproducible and reduced in patients with coronary artery disease. Endothelial function in both coronary and systemic (IMA) arteries can now be measured noninvasively with the same imaging technique and promises novel insights into systemic and local factors affecting vascular health. (Circ Cardiovasc Imaging. 2016;9:e003954. DOI: 10.1161/CIRCIMAGING.115.003954.)

Key Words: atherosclerosis ◼ coronary artery disease ◼ endothelium ◼ magnetic resonance imaging ◼ vasodilation

In response to certain stresses, the healthy endothelium releases nitric oxide (NO), which induces local vascular smooth muscle dilation, inhibits platelet aggregation, attenuates inflammation, and decreases cellular proliferation.1 Endothelial dysfunction is characterized by decreased NO bioavailability, occurs early in the development of atherosclerosis, predicts adverse cardiovascular events,2 and is a potential target for medical interventions.2–7 Peripheral arteries are more accessible to study than the coronary arteries, but there is only a modest correlation between coronary and peripheral endothelial function measures,8,9 and the coronary vascular bed differs significantly from the systemic vasculature.9,10 Patients with coronary artery disease (CAD), in fact, manifest a paradoxical coronary artery vasoconstrictor response to endothelial-dependent stressors that normally cause vasodilation,11 whereas the brachial arteries of patients with CAD vasodilate less in response to endothelial dependent stressors than do those of healthy individuals.8,12 A recent meta-analysis identified a variable association between cardiovascular events and endothelial dysfunction, depending on whether a central or peripheral vascular bed was studied.13 Moreover, studies that compared vasoreactivity of the brachial and the coronary vascular beds were performed using different imaging modalities at different times.8 The measurement of endothelial function in systemic arteries that do not develop atherosclerosis, like the brachial artery, provides information about systemic vascular health, whereas coronary artery endothelial function (CEF) measures offer insights into the contributions of systemic and local coronary factors including the presence of coronary atherosclerosis. Ideally, endothelial function would be assessed in systemic and coronary arteries at the same time using the same endothelial-dependent stressor and imaging technology.13

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Recently, the combination of new noninvasive 3T coronary magnetic resonance imaging (MRI) methods and isometric handgrip exercise (IHE), an endothelial-dependent stressor, has been reported as a means to noninvasively and reproducibly quantify CEF.11,14–16 Furthermore, we recently demonstrated in vivo that the coronary artery response to IHE is reproducible and primarily NO-mediated because it is blocked by the NO synthase inhibitor monomethyl-l-arginine (l-NMMA) in healthy subjects.17

The internal mammary artery (IMA) is a systemic vessel that rarely develops atherosclerosis,18 is often used as a coronary artery graft, and has been used to study systemic endothelial function.19–20 Because the right and left IMA are visible in many coronary MRI, especially in axial planes, which also intersect the right coronary artery (RCA), we posited that measurements of the vasodilator and blood flow (BF) responses of the IMA could be obtained at the time of CEF measures and serve as an index of systemic endothelial function. We tested the hypotheses that 1) the IMA vasoreactive response to IHE is NO dependent, ie, the response can be blocked by l-NMMA, a NO synthase inhibitor and thus reflects NO-mediated endothelial function, 2) the IMA-IHE response is reproducible, 3) IMA endothelial function is reduced in patients with CAD compared with that of healthy subjects, and 4) among patients with CAD, the endothelial-dependent IMA vasoreactive response to IHE differs from the coronary response.

Methods
Participants
The protocol was approved by the Institutional Review Board of Johns Hopkins Medicine and complies with the Declaration of Helsinki. An Investigational New Drug Application was obtained from the Food and Drug Administration (#119574) for the administration of l-NMMA. All participants provided written informed consent. All subjects were outpatients with no known contraindications to MRI. Healthy subjects were those without history of CAD and for those >50 years with an Agatston coronary artery calcium score <10 by computed tomography or an exercise stress test negative for inducible ischemia. Patients with CAD were individuals with stable CAD documented on prior coronary x-ray angiography or computed tomographic angiography (stenosis of 30–70%). The segment of the coronary artery selected for MRI measures of area, velocity, and BF in patients with CAD had no >30% luminal stenosis.

Study Protocol
All participants underwent MRI in the morning after an overnight fast (>8 hours) and before administration of any prescribed vasoactive medications. MRI were taken perpendicular to a proximal or mid well-visualized linear segment of the IMA and native RCA that had not undergone prior intervention or had a significant stenosis. To ensure that slice orientation was perpendicular to the coronary and IMA, double oblique scout scanning was performed as previously reported.19 Both the RCA and an IMA were imaged for cross-sectional area (CSA) or flow velocity (FV) in cross-section during the same single breath-hold cine sequence although in some instances they were imaged in different sequences if not parallel to one another. Either the left or the right IMA was chosen, depending on which was parallel to the RCA segment. All acquisitions were performed during a period of minimal motion in the cardiac cycle visually determined from cine axial images. Baseline images were acquired at rest for cross-sectional RCA and IMA area and velocity measurements, followed by repeat imaging at the same anatomic locations during 4 to 7 minutes of continuous exercise at 30% of maximum grip strength.15 IHE was performed using an MRI-compatible handgrip dynamometer (Stoelting, Wood Dale, IL) under direct observation and coaching by a research nurse. Heart rate and blood pressure were measured throughout during the study using a noninvasive and MRI-compatible ECG and blood pressure monitor (In vivo; Precess, Orlando, FL). The rate pressure product (RPP) was obtained as previously described.11 The MRI end points were CSA, FV, and BF measures as described above.

To test the study hypotheses, we conducted 2 protocols outlined here:

1. Role of NO in the IMA vasomotor response to IHE: the l-NMMA protocol:
   To assess whether the IHE-induced vasoreactive changes of the IMA are NO-mediated and hence endothelial dependent, 8 healthy individuals underwent IMA imaging before and during IHE while intravenous saline (placebo) was infused. After 10 minutes of post-IHE recovery, without repositioning, each subject then received an intravenous infusion of the NO synthase inhibitor, l-NMMA, at a dose of 0.3 mg/kg per minute, as previously described.21 A new set of baseline IMA images was obtained 5 to 10 minutes after initiation of the l-NMMA infusion. Each subject then performed a second IHE during continued l-NMMA infusion, and IMA imaging was repeated at the same location. The entire l-NMMA infusion lasted 15 to 22 minutes, and the entire MRI-CEF-l-NMMA protocol lasted ≥60 minutes.

2. IMA and RCA MRI vasomotor responses to IHE in healthy volunteers and patients with CAD:
   Healthy subjects (n=26) and patients with CAD (n=25) were consecutively enrolled, and both the RCA and an IMA were imaged in cross-section for CSA and FV at rest, followed by repeat imaging during IHE. Interobserver reproducibility analysis of IMA measurements (CSA, FV, and BF) was performed in a randomly selected subset of both healthy volunteers (N=6) and patients with CAD (n=5), and none from the subset were excluded from analysis.

Magnetic Resonance Imaging
A commercial human 3.0 Tesla whole-body magnetic resonance scanner (Achieva, Philips, Best, NL) with a 32-element cardiac coil for signal reception was used. Cross-sectional anatomic and FV encoded spiral MRI were obtained using single breath-hold cine sequences.22 MRI parameters for anatomic imaging were repetition time, 18 ms; echo time, 2.1 ms; radio frequency excitation angle, 20°; acquisition window, 13 ms; 17 to 21 spiral interleaves/cine frame; spatial resolution, 0.89x0.89x8.0 mm; and breath-hold duration, ≥14–24 s. MRI parameters for the velocity measurements were repetition time, 40 ms; echo time, 3.5 ms; radio frequency excitation angle, 20°; acquisition window, 33 ms; 9 to 11 spiral interleaves/cine frame; spatial resolution, 0.8x0.8x8.0 mm3; velocity encoding, 35 to 75 cm/s; and breath-hold duration, 14 to 24 s.

Image Analysis
Baseline and IHE stress images were analyzed for RCA and IMA CSA using semiautomated software (Cine version 3.15.17, General Electric, Milwaukee, WI). A circular region-of-interest around the RCA and the IMA was traced during a period of least coronary motion over 3 sequential images. The 3 values (measured in mm2) were then averaged. The computer algorithm used an automated full width half maximum algorithm for CSA measurements. For flow measurements, velocity measurements of the same baseline and IHE stress...
RCA and IMA images were made using commercially available software (QFLOW Version 3.0, Medis, The Netherlands). A region of interest was traced using semiautomated software around a cross-sectional RCA to obtain peak diastolic coronary FV and of the IMA for peak systolic FV (mean velocity of lumen pixels at peak flow), both referred to as FV hereafter. Velocity was measured in centimeters per second and coronary and IMA BF (in mL/min) were calculated and converted to units of milliliter per minute using the adapted equation: CSA × peak FV × 0.3. Segments with poor image quality (blurring because of artifact/patient motion) on either baseline or stress exams were excluded from analysis.

Statistics
The data were tested for normality using the Shapiro–Wilks test. Parametric (Student’s t test) and nonparametric testing (Wilcoxon signed-rank test for paired data and Wilcoxon rank-sum test for non-paired data) were used when appropriate for normally distributed and not normally distributed data, respectively, to compare the response to IHE from baseline measurements in the RCA and IMA of healthy subjects and patients with CAD for area, velocity, and flow measurements. A paired t test was used to compare IHE-induced IMA changes during placebo to those during l-NMMA infusion. For comparisons among 4 groups, 1-way ANOVA and Kruskal–Wallis were used for parametric and nonparametric comparisons, respectively, with Bonferroni adjustment used for pair-wise comparisons. Linear regression analysis was performed to assess inter-reader reproducibility, and the mean differences were displayed with Bland–Altman regression analysis was performed to assess inter-reader reproducibility, and the mean differences were displayed with Bland–Altman analysis (Figure 1). Magnified cross-sectional image of the RCA and the IMA (shown by green and red boxes, respectively) at rest (C) and during IHE (D). Magnified flow velocity image of the IMA in the same subject is shown at rest (E) and during IHE (F) in systole. Magnified coronary flow velocity image of the RCA in the same subject is shown at rest (G) and during IHE (H) in diastole. The signal phase is proportional to flow velocity with the darker pixels in the velocity phase contrast images during IHE indicating higher velocity in the caudal direction of the IMA and RCA.

Results
All subjects completed the study. An example of typical changes seen in area and velocity with IHE in a healthy volunteer is shown for the IMA and RCA in Figure 1. Modest increases in CSA and larger increases in blood flow (shown by darker pixels in the velocity encoded phase contrast images indicating increased velocity in the caudal direction) occur during IHE in healthy subjects.

Role of NO in Mediating the IMA Response to IHE

Subject Characteristics and Hemodynamic Effects of l-NMMA
Eight healthy subjects underwent IMA imaging with the l-NMMA protocol (age: 30 ± 4 years). The baseline RPP increased significantly with IHE (P < 0.0001) and returned to baseline during the recovery period (P = 0.8). During l-NMMA infusion, mean RPP was not different from that before l-NMMA. The increase in RPP during IHE was similar in the absence and presence of l-NMMA.

l-NMMA Infusion Blocks the Vasodilatory Response of the IMA to IHE
The IMA responses to IHE in 8 healthy subjects during placebo and l-NMMA infusion are presented in Figure 2B. There is an ≈15% increase in CSA, 30% increase in coronary FV, and ≈50% increase in coronary BF during placebo infusion (Figure 2B). IMA CSA increased from a baseline of 8.5 ± 0.6 mm² to 9.8 ± 0.7 mm² (P < 0.001) during IHE. However, there was no significant increase in IMA CSA when IHE was repeated during l-NMMA infusion (second baseline: 9.0 ± 0.8 mm² versus l-NMMA-IHE: 9.2 ± 0.8 mm²; P = 0.2). In relative terms, %CSA change with IHE was 15.4 ± 2.2% with placebo versus 2.3 ± 1.3% with l-NMMA (P < 0.001).

l-NMMA Infusion Blocks the IMA Increase in Blood Velocity and Flow With IHE
Peak systolic velocity and BF in the IMA significantly increased with IHE during placebo infusion. Velocity increased from a baseline of 21.4 ± 3.3 to 27.4 ± 4.2 cm/s (P < 0.01) with IHE, whereas BF increased from a baseline of 50.2 ± 3.1 to 74.1 ± 4.7 mL/min (P < 0.0001) with IHE during placebo. l-NMMA infusion did not change baseline IMA flow but completely blocked the IHE-induced increases in velocity and flow (Figure 2). This suggests that the normal vasoactive IMA response to IHE is predominantly NO mediated.

Reproducibility of IMA Measurement
Changes of the IMA CSA, FV, and BF during IHE were analyzed by 2 observers (M.I. and A.H.). The results strongly correlated for the %CSA, %FV, and %BF change with IHE (Figure 3A through 3C). The Bland–Altman analysis (Figure 3D and 3F) and intraclass correlation coefficients for %CSA, %FV, and %BF change with stress in the IMA (intraclass correlation coefficient=0.89, 0.97, and 0.93, respectively) indicated excellent confidence of agreement and little variability between the 2 measures.
IMA and RCA MRI Vasomotor Responses to IHE in Healthy Volunteers and Patients With CAD

Subject Characteristics

We compared the responses of 26 consecutive healthy subjects (age: 45±3.5 years) and 25 patients with CAD (61±1.5 years). The study subject characteristics are summarized in the Table. To age-match healthy subjects with patients with CAD, the results from an older subset of the original healthy individuals (n=12; 61±3 years) were also compared with those from the patients with CAD (61±1.5 years; P=NS), and those data appear in the Data Supplement section.

Hemodynamic Effects of IHE in Healthy Volunteers and Patients With CAD

IHE induced significant and similar hemodynamic changes in healthy subjects and patients with CAD. In the healthy group, we observed a mean 35.4±4.6% increase in RPP, which was not different than the mean 28.7±3.9% RPP change with IHE in patients with CAD (P=0.3 versus healthy subjects).

Coronary and IMA Area Changes

Both the RCA and IMA in healthy subjects dilated significantly in response to IHE (P<0.001 and P<0.0001 from baseline, respectively; Figure 4). In contrast, the RCA in patients with
Dyslipidemia (%) 2 (7) 2 (17) 24 (96)

ACE-inhibitor (%) 1 (4) 1 (8) 15 (60)

Statin (%) 2 (7) 2 (17) 24 (96)

Diabetes mellitus (%) 0 0 3 (12)

ACE indicates angiotensin-converting enzyme; ASA, aspirin; CABG, coronary artery bypass graft; CAD, coronary artery disease; HTN, hypertension; MI, myocardial infarction; and PCI, percutaneous intervention.

CAD did not vasodilate in response to IHE (P=0.4 for CSA compared with baseline), as we previously reported.11,15 However, the IMA did vasodilate with IHE in patients with CAD (P<0.0001 from baseline) although significantly less than that in healthy volunteers (%IMA change from baseline: 16.4±2.5% in healthy versus 9.1±1.6% in patients with CAD; P=0.02; Figure 4B). The RCA CSA response to IHE was significantly less than the IMA response in patients with CAD (patients with CAD: RCA %CSA change with IHE: −0.9±1.7% versus IMA %CSA change with IHE: +9.1±1.6%; P<0.001; Figure 4B) but not in healthy subjects (healthy: RCA %CSA change with IHE: 11.7±2.0% versus IMA %CSA change with IHE: 16.4±2.5%; P=NS).

Coronary and IMA Velocity and BF Measures
For the IMA, both FV and BF increased significantly with IHE in healthy individuals (P<0.001). In contrast, in patients with CAD, we observed an attenuated but significant increase in IMA BF with IHE from baseline (P<0.001). The IMA changes in patients with CAD were less than those of healthy individuals (P<0.001 IMA versus healthy IMA; Figure 5B). Consistent with prior reports, the RCA vasoreactive responses were characterized by a significant increase in FV and BF in healthy subjects but not in CAD patients with IHE (Figure 5B and 5C).11,15,16 When comparing the 2 vascular beds, the coronary BF response was less than the IMA response in patients with CAD but did not reach statistical significance (P=0.07; Figure 5C). When the results on healthy subjects and patients with CAD were combined into a single group, there was a statistically significant relationship between IMA and RCA IHE responses (Figure 6). However, the correlations were not generally significant when the groups were considered separately, suggesting that the IMA–RCA correlation is highly influenced by group differences between healthy subjects and patients with CAD rather than by a close fundamental relationship between IMA and RCA responses.

Discussion
Abnormal vasomotor responses of systemic and coronary arteries to endothelial-dependent stressors predict subsequent cardiovascular events but the direction, magnitude, and prognostic value of the responses often differ between systemic and coronary arteries.8,9,25,26 Because systemic and coronary endothelial function measures are typically obtained at different times, with different endothelial-dependent stressors, and assessed with different techniques,8,14 it is difficult to know whether different responses between systemic and coronary arteries represent true disparities in local vascular biology or are simply because of differences in the stimulus, means of detection, and/or conditions at the time of study. In this article, we describe the first noninvasive means to simultaneously assess endothelial function in systemic and coronary circulations. The measures of systemic (IMA) and coronary (RCA) endothelial function are obtained at the same time, in response to the same stimulus, and detected with the same imaging technology. We demonstrate that the IMA response to IHE is indeed predominantly NO-mediated, reproducible between observers, differs between healthy subjects and those with CAD, and differs from the response of the coronary arteries in patients with CAD.

Role of NO in Mediating the IMA Response to IHE
We recently reported that the coronary response to IHE is measurable with MRI11,15,16 and mediated by NO in healthy individuals.17 Because it was not previously known whether the IMA response to IHE is NO-mediated,27 we report here that the NO synthase inhibitor l-NMMA abolishes the normal
IMA vasodilatory and BF IHE responses in healthy subjects (Figure 2). Our results are consistent with prior studies indicating that l-NMMA blocks ≈70% of the brachial artery macrovascular vasodilatory response in healthy subjects.28,29 Likewise, the microvascular peripheral response during IHE is also blocked by l-NMMA infusion.28 Together these findings in healthy subjects demonstrate that IHE is a predominantly NO-mediated endothelial dependent stressor for both the coronary and systemic circulations. Our assumption that NO also acts in the CAD population is reasonable although not directly demonstrated. We did not administer l-NMMA to patients with CAD because l-NMMA reduces NO synthesis and one would expect minimal or no vascular effect in an already severely NO-deficient state like CAD, especially when monitoring the coronary vasoactive dilatory or flow effects of IHE that are already absent in patients with CAD. In addition, l-NMMA may pose risk in patients with CAD. Thus, continuous IHE as described here can be used with MRI or potentially other noninvasive imaging modalities to simultaneously probe coronary and systemic NO-mediated endothelial function.

IHE-Induced Vasoreactivity of the IMA Is Reduced in Patients with CAD Compared With Healthy Subjects

We observe a significant reduction of IHE-induced vasoreactivity of the IMA in patients with CAD compared with that of healthy subjects (Figures 4 and 5). These observations are in line with prior in vitro studies10,30 as well as in vivo studies showing an impaired IMA vasodilatory response to acetylcholine infusion in patients with CAD when compared with healthy volunteers.

Figure 5. Peripheral and coronary endothelial function in healthy volunteers and patients with CAD-flow change. A, Protocol diagram illustrating MRI study. Summary results for mean peak systolic velocity changes in the IMA and peak diastolic velocity changes in the RCA (B) and for mean blood flow changes with IHE (C) during IHE for healthy subjects (blue) and patients with CAD (red). Analysis performed with Kruskal–Wallis testing with Bonferroni adjustment for pair-wise comparisons. CAD, coronary artery disease; IMA, internal mammary artery; IHE, isometric handgrip exercise; MRI, magnetic resonance imaging; and RCA, right coronary artery.

Figure 6. Relationship between coronary and internal mammary artery endothelial function. Individual results for IMA and RCA responses to IHE for CSA (A), flow velocity (B), and blood flow (C) changes in healthy subjects (blue) and patients with CAD (red). When all participants were combined into a single group there was a statistically significant relationship between IMA and RCA responses (although the significance of the CSA relationship depended on 1 point remote from the others). However, the correlation between IMA and RCA responses were not significant for each group considered alone, suggesting the overall correlation was primarily related to group differences between healthy subjects and patients with CAD rather than a close fundamental relationship between IMA and RCA responses. CAD, coronary artery disease; CSA, cross-sectional area; IMA, internal mammary artery; IHE, isometric handgrip exercise; and RCA, right coronary artery.
controls. Berkenboom et al showed that selective infusion of the IMA with L-arginine, a NO precursor, reversed the impaired IMA response to acetylcholine in patients with CAD, suggesting that decreased NO plays a critical role in the pathogenesis of the diminished IMA response in those patients. The IMA vasoreactive response generally resembles the brachial response in direction and magnitude in diseased states.

Systemic Versus Coronary Endothelial Function: Differences in Vascular Properties
Among patients with CAD, we observe that coronary arteries fail to dilate and sometimes vasoconstrict in response to IHE, whereas the IMA responds with reduced vasodilation, similar to the attenuated brachial response during forearm cuff occlusion. One obvious difference between the coronary and IMA vascular beds is that the latter does not develop atherosclerosis while the coronary arteries do. Furthermore, the vasoreactive responses of coronary and systemic vessels were previously compared, albeit at different time points and imaging modalities, and the correlation between systemic and coronary endothelial function was not strong. When the endothelial responses of the IMA and RCA were compared for individual subjects (Figure 6), the correlation was not significant for healthy subjects or for patients with CAD alone although significant when combined. One prior study showed that intraarterial infusion of L-arginine did not affect acetylcholine-induced vasodilation in the coronaries of healthy individuals and patients with CAD, whereas it augmented the acetylcholine-induced increase in forearm BF in the 2 groups. Thus, the response to acetylcholine infusion differed between coronary and systemic arteries suggesting that mechanisms for vasodilation may vary between the vascular beds. Differences in vascular properties between coronary and systemic vessels may be because of a variable amount of NO production and/or bioavailability. Finally, although atherosclerosis is often regarded as a systemic process, the often-disparate vasoreactive responses of coronary and systemic arteries suggest differences in local milieu. Prior observations that coronary arteries display a heterogeneous coronary endothelial response depending on the degree of atherosclerotic disease suggest that local factors may contribute to local atherosclerotic plaque formation despite the exposure of all coronary segments to identical systemic factors. Therefore, the study of these 2 vascular territories may shed important insights into their relationship and how external and local factors may influence them in different ways. Because endothelial function is often considered a barometer of vascular health and responds rapidly to protective strategies, we postulate that this MRI technique could be used in the future to guide therapy (eg, lowering low-density lipoprotein not to an low-density lipoprotein number but until endothelial function improves), predict future events in at risk populations, and, importantly, to rapidly test the ability of new strategies to improve systemic and coronary vascular health.

Limitations
Sample size is relatively modest in the L-NMMA study but yet large enough to show highly statistically significant responses. Although the RCA was the only coronary artery studied here with the IMA, it is possible to acquire coronary endothelial function measures of the left coronary artery system during the same IHE session. Although phasic BF changes during the cardiac cycle may differ between RCA and left anterior descending coronary artery (LAD), the latter does not lie orthogonal to the axial plane like the IMA and RCA, and thus, the LAD would require an additional breath-hold acquisition. Local coronary factors likely play a major role in influencing macrovascular regional CEF, but we cannot exclude the possibility, based on the current data alone, that differences in distal NO production in the microvasculature could affect shear stress and thereby proximal coronary artery changes to IHE. Regardless, this new approach offers a reproducible measure of NO-mediated endothelial function in coronary and systemic vascular territories concurrently with the same stressor, under the same conditions and detected with the same imaging technology. In future studies, it would be useful to compare these measures of systemic endothelial function derived from IMA to those of other, more commonly studied arteries (ie, brachial and femoral) and to determine the extent to which these measures are associated with future cardiovascular events.

Conclusions
In summary, we report here the first noninvasive approach for concurrently measuring systemic and coronary vascular endothelial function. The IMA-IHE response is predominantly NO dependent, can be measured noninvasively with MRI simultaneously with CEF measures, and is reproducible both in healthy volunteers and patients with CAD. Importantly, the IMA-IHE response in patients with CAD differs significantly from that in healthy subjects. This noninvasive approach promises a more complete assessment of vascular health than measures of endothelial function in a single vascular territory and will enable the systematic study of interventions designed to improve endothelial function over time.

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Disclosures
None.

References

**CLINICAL PERSPECTIVE**

Endothelial dysfunction is characterized by decreased nitric oxide bioavailability, occurs early in the development of atherosclerosis, predicts adverse cardiovascular events, and is a potential target for medical interventions. Thus, endothelial function is considered a barometer of vascular health. Coronary artery and peripheral artery endothelial function are often, however, weakly correlated, and it is not clear whether the heterogeneous responses are because of biological differences between the vascular territories or because of the dissimilar techniques and stressors used to obtain the measures. This study demonstrates, for the first time, that noninvasive MRI measures of coronary artery and internal mammary artery (IMA) endothelial function can be assessed at the same time, in response to the same stressor (handgrip exercise), and detected using the same imaging modality. Moreover, the IMA responses are reproducible and primarily mediated by nitric oxide. Thus, it is now possible to simultaneously assess systemic vascular health in vessels that do not typically develop atherosclerosis (IMA) along with coronary vascular health in vessels that do develop atherosclerosis and to do so noninvasively. Future trials evaluating the impact of new therapeutic interventions on both peripheral and coronary vascular health can use this as a noninvasive, reproducible means to quantify changes in nitric oxide-mediated endothelial function and thereby enhance assessment of the impact of these interventions on cardiovascular risk, pathogenesis, and disease progression in patients with coronary artery disease.
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Supplemental Material

IMA and RCA MRI vasomotor responses to IHE in CAD patients and age-matched healthy subjects

Subject Characteristics
In order to age-match healthy subjects with CAD patients, an older subset of the original healthy individuals (n=12: age-matched healthy subjects 61±3 years (range 50-83 years)) was compared to the CAD patients (n=25: 61±2 years, (range 52-76) p=NS).

Coronary and IMA area changes measures
Both coronary arteries and IMA in healthy subjects dilated significantly in response to IHE (p<0.001 and p=0.0001 vs baseline, respectively). The RCA area did not significantly change in response to IHE (p=0.4 vs baseline; Suppl Fig1) in CAD patients. In contrast, the IMA vasodilates with IHE in CAD patients (p<0.0001 vs baseline) although less as compared to that observed in healthy volunteers (% IMA change from baseline 15.9±2.9% in healthy vs 9.1±1.6% in CAD patients; p=NS; Suppl Fig 1).

Coronary and IMA velocity and blood flow measures
For the IMA, both FV and BF increased significantly with stress in healthy individuals (p=0.04; and p<0.01; vs baseline). In contrast, in CAD patients, although there was a mild increase in FV and a significant increase in BF with IHE in the IMA (p<0.0001 vs. baseline for both), the degree of change was significantly less than that seen in healthy individuals. In CAD patients, the IHE-induced change in flow was higher for the IMA compared to the RCA (%;p=0.04) (Suppl Fig1).

Suppl Fig.1. Peripheral and Coronary Endothelial Function in Age-Matched Healthy Volunteers and Patients with CAD.
A) Summary results for mean area changes in the Internal Mammary Artery (IMA) and Right Coronary artery (RCA) during Isometric Handgrip Exercise (IHE) (as % of baseline values) for age matched healthy volunteers and patients with CAD. Error bars indicate standard error of the
mean. B) Summary results for mean peak systolic velocity changes in the IMA and peak diastolic velocity changes in the RCA during IHE (as % of baseline values) for healthy volunteers and patients with CAD. Error bars indicate standard error of the mean. C) Summary results for mean blood flow changes in the IMA and the RCA during IHE (as % of baseline values) for healthy volunteers and patients with CAD. Error bars indicate standard error of the mean. Analysis performed with Kruskal-Wallis testing with Bonferroni adjustment for pair-wise comparisons.
Suppl Fig. 1

**A**

Bar chart showing % Change from baseline in Area. Group A: healthy volunteers N=12, Group B: CAD patients N=25. Comparisons: P<0.001.

**B**

Bar chart showing Velocity. Group A: healthy volunteers N=12, Group B: CAD patients N=25. Comparisons: P=0.04, P<0.001.

**C**

Bar chart showing Flow. Group A: healthy volunteers N=12, Group B: CAD patients N=25. Comparisons: P<0.001, P=0.04, P=0.01.