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## MRI and Characterization of Atherosclerotic Plaque Emerging Applications and Molecular Imaging

Robin P. Choudhury, Valentin Fuster, Juan J. Badimon, Edward A. Fisher, Zahi A. Fayad

**Abstract**—Noninvasive high-resolution magnetic resonance has the potential to image atherosclerotic plaque and to determine its composition and microanatomy. This review summarizes the rationale for plaque imaging and describes the characteristics of plaque by use of existing MRI techniques. The use of MRI in human disease and in animal models, particularly in rabbits and mice, is presented. Present and future applications of MRI, including real-time vascular intervention, new contrast agents, and molecular imaging, are also discussed. (*Arterioscler Thromb Vasc Biol.* 2002;22:1065-1074.)

**Key Words:** MRI ■ atherosclerosis ■ plaque ■ molecular imaging ■ mice

The introduction of percutaneous arteriography by Fariñas<sup>1</sup> in 1941 and selective coronary arteriography by Sones<sup>2</sup> in 1957 made clinical imaging of atherosclerosis possible. Arteriography provides useful anatomic information that has been used to guide decisions about treatment and to enable the delivery of therapy in the case of percutaneous interventions.<sup>3-5</sup> However, arteriography images only the vessel lumen and the silhouette of lesions that impinge on the lumen. Atherosclerosis can develop in the arterial wall and be accommodated by outward (or positive) arterial remodeling.<sup>6,7</sup> At sites of positive remodeling, lumen caliber may be unaltered or minimally altered and, therefore, not detected by arteriography. The importance of this has been highlighted in angiographic studies demonstrating that nonsevere stenoses are more often associated with acute coronary events than are severe coronary stenoses.<sup>8,9</sup> From a pathological perspective, plaques with large lipid cores and thin fibrous caps are more prone to rupture, leading to thrombosis and vascular events, than are plaques with small securely contained lipid cores and thick caps.<sup>10,11</sup> The present challenge is to develop imaging technology capable of characterizing atherosclerosis, particularly in human coronary arteries. This may allow identification (and treatment) of plaques that are at risk of future rupture and thrombosis.<sup>12</sup>

### See page 1064

Numerous imaging modalities, including thermography, near infra-red spectroscopy, Raman spectroscopy, ultrafast CT, and ultrasound have been applied to the characterization of plaque and are reviewed in detail elsewhere.<sup>13-15</sup> However, MRI has the greatest potential for clinical application. Magnetic resonance (MR) is well suited to this role because it is noninvasive, does not involve ionizing radiation, can be repeated serially, and provides high-resolution images of the vessel wall and lumen.

Atherosclerosis usually develops silently over many years, although significant lesions are commonly present as early as the second decade.<sup>16</sup> Lipid-lowering drugs have demonstrated efficacy and safety in the primary prevention of the complications of atherosclerosis,<sup>17,18</sup> but the present guidelines do not include the presence of subclinical atherosclerosis in decisions about therapy.<sup>19</sup> In the future, risk stratification, which includes noninvasive identification and characterization of atherosclerosis, may direct the type and intensity of treatment in individual patients, even before clinical disease has been allowed to manifest itself.

Our expanding knowledge of plaque composition, biology, and behavior demands that imaging modalities provide quantitative and qualitative information about the plaque.<sup>10,20-23</sup> The present review will summarize the rationale for plaque

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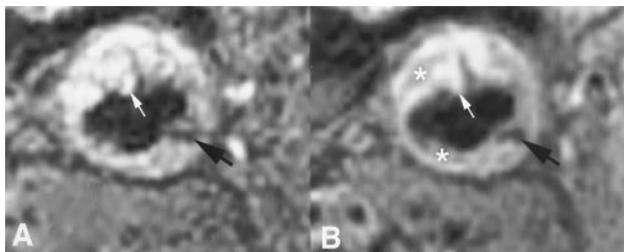
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**Figure 1.** A 67-year-old male with atherosclerotic disease in the common carotid artery imaged with in vivo high-resolution MR. Multicontrast (T1W, PDW [not shown], and T2W) MR images are obtained to characterize all the plaque components. A, T1W image. B, T2W image. A complex lesion is detected with a fissure (at 12 o'clock). Black arrows indicate calcium; asterisk, lipid deposits; and white arrows, thrombus.

imaging, provide a technical overview of MRI, and describe the characteristics of plaque by use of existing MRI techniques. Present and future applications of MRI, including targeted contrast and molecular imaging, will also be discussed.

### Principles of MRI

MRI has emerged as the potential leading noninvasive in vivo modality for atherosclerotic plaque imaging in experimental animals<sup>24–30</sup> and in humans.<sup>26,31–35</sup> The principles of MRI are described in detail elsewhere.<sup>36–38</sup> In brief, MR characterizes plaque on the basis of the biophysical and biochemical properties of its different components. Representative MR images can be seen in Figures 1 through 4.

During the examination, the subject is positioned in a high-external-static magnetic field (usually 1.5 T for human studies; see Tables 1 and 2), which aligns the protons in the body. Thus far, the application of an external static magnetic field to the spins will result in a net magnetization that is parallel to the applied field. This longitudinal magnetization is not detected. Instead, the longitudinal magnetization must be converted into a transverse magnetization, perpendicular to the applied static field, before it can be detected. This conversion can be accomplished by the application of a time-varying electromagnetic radiofrequency (RF) pulse, applied at the resonance frequency. The protons can then absorb that energy. The transverse magnetization created does not remain in the transverse plane indefinitely. After the RF pulse is turned off, 3 events begin to happen simultaneously: (1) The absorbed RF energy is retransmitted (at the resonance frequency). This is the “MRI signal.” (2) The excited spins begin to return to the original equilibrium longitudinal magnetization. The rate at which the recovery occurs is deter-

mined by the spin-lattice relaxation time (T1). Fortunately, the T1 relaxation times vary among tissue types, providing a highly useful means of generating image contrast. (3) Initially, in phase, the excited protons begin to dephase at a rate characterized by the spin-spin relaxation time (T2). The T2 relaxation times also vary with tissue type, providing another means of generating tissue contrast. The resulting “MRI signal” is detected by receiving RF coils.

Images in which most of the contrast between tissues is derived from differences in tissue T1 are termed T1-weighted (T1W) and, analogously, T2-weighted (T2W) images. A proton density-weighted (PDW) image is obtained when the differences in contrast are proportional to the density of water and fat protons within the tissue.

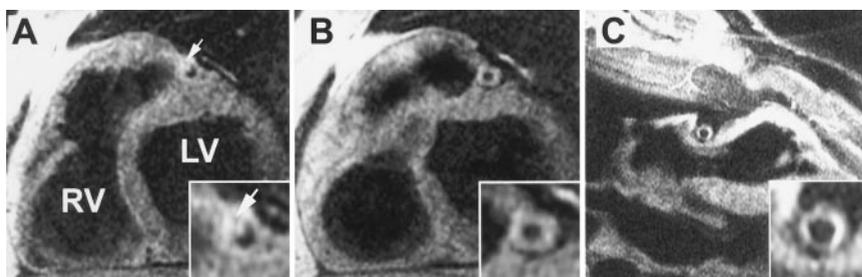
Three additional mutually perpendicular magnetic fields (gradient fields) are applied during MRI: 1 to select the slice and 2 to encode spatial information. As a result, each voxel within the imaged tissue is uniquely identified.

### Determination of Plaque Components With MR

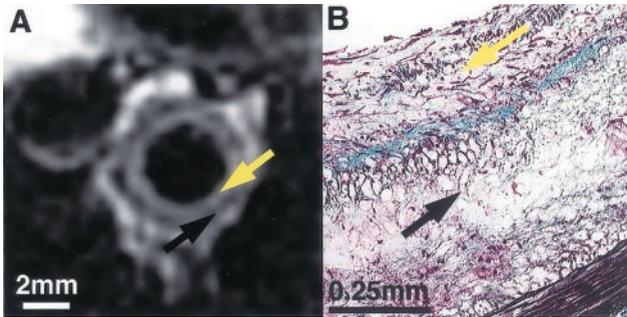
Atherosclerotic plaques are of heterogeneous composition. Angiographic and pathological<sup>21,23</sup> studies have determined the plaque types at greatest risk of acute rupture or erosion. In human coronary arteries, location,<sup>39</sup> geometry,<sup>10,40</sup> and composition<sup>10,40–43</sup> are all useful indicators of vulnerability. In particular, the presence of a large extracellular lipid core, thin fibrous cap, and inflammatory cell infiltrate indicates plaques at risk.<sup>10,44</sup> Can MRI rise to the challenge of discerning these factors?

### Plaque Characterization by Non-Contrast-Enhanced MRI

In MRI, the emitted RF signal differs between the nuclei of different atoms and further varies according to the molecular environment of the nuclei. In this way, it is possible to obtain quantitative information about specific molecules of interest within a given tissue. In early MR studies of atherosclerosis, characterization was directed toward chemical shift imaging by use of the lipid signal.<sup>45,46</sup> These studies were designed to image plaque lipids with long T2 and short T1 relaxation times, similar to triglycerides. However, unlike periaortic fat, which is composed of fatty acyl triglycerides, the lipid components of the plaque are predominantly cholesterol, cholesteryl ester, and phospholipid,<sup>47</sup> whose MR characteristics are different from the fat of adipose tissue.<sup>48</sup> Furthermore, chemical shift imaging aimed at directly imaging lipid components of the plaque is intrinsically disadvantaged, because



**Figure 2.** In vivo MR black-blood cross-sectional images of human coronary arteries demonstrating a plaque presumably with deposition of fat (arrow, panel A), a concentric fibrotic lesion (panel B) in the left anterior descending artery, and an ectatic, but atherosclerotic, right coronary artery (panel C). RV indicates right ventricle; LV, left ventricle. Figure modified from Fayad et al.<sup>34</sup>

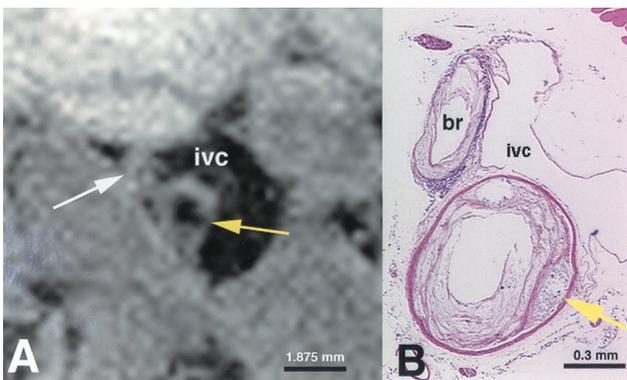


**Figure 3.** A, In vivo MR images (T2W) of rabbit abdominal aorta. B, Corresponding histopathological section (combined Masson elastin stain). MRI differentiates fibrotic (high-signal, yellow arrow) and lipid (low-signal, black arrow) components of the plaque. Figure modified from Helft et al.<sup>30</sup>

even in relative lipid-rich plaque, the signal from water predominates by  $\approx 10$ -fold.<sup>49,50</sup> For these reasons, recent studies have focused on MRI of water protons.<sup>50,51</sup>

By use of a combination of inherent MRI contrast generated in T1W, T2W, and PDW images (Table 1), it has been possible to determine plaque anatomy and composition in experimental animals,<sup>24,30</sup> in ex vivo specimens,<sup>33,50,52</sup> and in human carotid arteries (Figure 1)<sup>33,53</sup> and aortas in vivo.<sup>54</sup> Recently, atherosclerosis has also been identified, in vivo, in human coronary arteries.<sup>34,55,56</sup> These applications are discussed further below.

Toussaint et al<sup>33</sup> demonstrated that fibrous cap, lipid core, media, and adventitia could be distinguished by use of high-field/high-resolution MRI. Differences in water T2 contrast, ex vivo<sup>33,50</sup> and in vivo,<sup>33</sup> identified lipid core versus fibrous cap, normal media versus lipid core, and media versus adventitia. Compared with the fibrous cap or media, the atheromatous core is associated with a shortened water T2 and, therefore, appears dark compared with the adjacent cap and media, which appear bright on T2W images. Calcified



**Figure 4.** T1W MR image (magnified; see scale) of atherosclerotic lipid-rich complex plaque in an apoE-knockout (apoE-KO) mouse. MR pixel size is  $97 \times 97 \times 500 \mu\text{m}^3$ . At the top left of the abdominal aorta, an atherosclerotic small branch vessel (br) is seen by MR (panel A, white arrow) and by histopathology (panel B) in 21-month-old apoE-KO mouse. This lesion had a focal calcium deposit in abdominal aorta (yellow arrow) that appeared as a signal void (panel A) and was correlated with histopathological findings (panel B), as show by hematoxylin-eosin stain (original magnification  $\times 40$ ). Inferior vena cava (IVC) is shown at right of the abdominal aorta (A). Figure was modified from Fayad et al.<sup>27</sup>

areas of plaque do not generate appreciable signal because of the low water content, but they can be detected as areas of low signal (black) on T1W images.<sup>14,57</sup>

Characterization of plaque in vivo in humans has been achieved in the aorta and carotid artery.<sup>33,53</sup> Fayad et al<sup>54</sup> found good correlation of multicontrast MRI with aortic plaque quantification and characterization by using transesophageal echocardiography. Hatsukami et al<sup>53</sup> used a 3D multiple-overlapping thin-slab MR/multiple-overlapping thin-slice angiography/time-of-flight technique to image the fibrous cap of carotid arteries before endarterectomy. In their study of 22 patients undergoing carotid endarterectomy (with a best voxel size of  $254 \times 254 \times 1000 \mu\text{m}^3$ ), thick fibrous caps were seen as a dark band between the lumen (white) and the vessel wall (gray). The presence of a thin cap was inferred from the absence of any discernible dark band. Plaque rupture was identified in vivo by MRI in 8 of the 9 cases in which it was subsequently identified on the atherectomy specimens. Also, in human carotid arteries that were imaged in vivo, Yuan et al<sup>58</sup> have identified lipid core with sensitivity 85% and specificity 92% by using time-of-flight-based bright blood and spin-echo-based black blood multicontrast techniques. Although lipid-rich necrotic cores were typically hypointense with T2W, this was variable, and as reported previously,<sup>52</sup> the comparison of vessel wall appearances under different contrast weightings provided the greatest diagnostic yield.

The same authors have recently demonstrated the clinical significance of carotid plaque characterization.<sup>59</sup> In a case-control study of patients undergoing carotid endarterectomy, a recent (within 90-day) history of transient ischemic attack or stroke was strongly associated with the presence of thin or ruptured plaque identified preoperatively by MRI. The risk of recent ischemic neurological symptoms was increased by an impressive 23-fold in cases in which ruptured plaque was identified compared with a thick fibrous cap. These encouraging observations will pave the way for studies that prospectively examine plaque behavior.

### Coronary Artery Imaging

Recently, coronary arteries have been imaged in vivo by MRI (Figure 2).<sup>34,55,56,60</sup> Coronary imaging poses considerable technical difficulties. The coronary arteries are relatively small and have a tortuous and unpredictable course. In addition, to obtain MR images, cardiac and respiratory motion must be overcome. Use of MR navigator echoes that assess cardiac or diaphragmatic position accounts for movement and eliminates the time constraint imposed by imaging in a single breath-holding, as shown in a recent multicenter study of coronary MR angiography.<sup>61</sup> This provides longer effective image acquisition to enable submillimeter spatial resolution (Botnar et al<sup>55</sup>). Botnar et al<sup>60</sup> have further refined their 3D coronary wall imaging technique by the application of a local inversion technique, improving contrast between lumen and vessel wall in a series of normal subjects and attaining a resolution of  $0.66 \times 0.66 \times 2 \text{ mm}^3$ .<sup>60</sup> Current coronary MRI techniques have limited spatial resolution mainly because of the available signal-to-noise ratio. One way to increase the signal-to-noise ratio directly is to improve the

**TABLE 1. MRI Parameters in Selected Cited Studies**

Species	Setting	Magnet, T	Contrast Weighting	TR/TE, ms/ms	Voxel, $\mu\text{m}$	Time	Reference
Xenopus	In vivo embryo	11.7	3D-spin echo-T1W	400/21	27 $\times$ 16 $\times$ 16	3 h 45 min	Louie, 2000 <sup>133</sup>
Mouse	In vivo tumor	1.5	T1W, T2W	na	300 $\times$ 300 $\times$ 700	3–7 min	Weissleder, 2000 <sup>132</sup>
Mouse	Ex vivo tumor	7.1	T1W, T2W	na	39 $\times$ 39 $\times$ 39	5.5 h	Weissleder, 2000 <sup>132</sup>
Mouse	Ex vivo brain	9.4	T1W	200/4	40 $\times$ 40 $\times$ 40	7 h	Sipkins, 2000 <sup>137</sup>
Mouse	In vivo aorta	9.4	T2W	2000/30	48 $\times$ 48 $\times$ 500	17 min	Fayad, 1998 <sup>27</sup>
			PDW	2000/13			
Rabbit	In vivo aorta	1.5	T2W	2300/60	35 $\times$ 35 $\times$ 3000	70 min	Heftt, 2001 <sup>30</sup>
			PDW	2300/17			
Pig	In vivo coronary	1.5	T2W	2 RR'/42	390 $\times$ 390 $\times$ 500	na	Worthley, 2000 <sup>28</sup>
			PDW	2 RR'/17			
Human	In vivo carotid	1.5	T2W	1 RR'/20 & 55	390 $\times$ 390 $\times$ 5000	na	Toussaint, 1996 <sup>33</sup>
Human	In vivo carotid	1.5	MOTSA-T1W	34; 22/2.9; 4.4	254 $\times$ 254 $\times$ 1000	1–5 min	Hatsukami, 2000 <sup>53</sup>
Human	In vivo coronary	1.5	T2W	2 RR'/25	500 $\times$ 1000 $\times$ 5000	na	Botnar, 2000 <sup>56</sup>
Human	In vivo coronary	1.5	T2W, BBI	2 RR'/40	460 $\times$ 460 $\times$ 3000	1 BH	Fayad, 2000 <sup>34</sup>

RR' indicates R-R' interval measured from electrocardiogram; MOTSA, multiple-overlapping thin-slab angiography; BBI, black blood imaging; BH, breath hold; na, data unavailable; TR, repetition time; TE, echo time.

receiver coils. This has been shown by Fayad et al,<sup>62</sup> who used a 4-element anterior phased-array coil that enabled in a series of patients in vivo coronary wall imaging at a resolution of 0.39 $\times$ 0.39 $\times$ 2 mm<sup>3</sup>. This in-plane resolution was found to be adequate in providing an accurate (measurement error <20%) assessment of the “normal” and diseased vessel wall area, as shown by numerical simulations and phantom measurements.<sup>63</sup>

Acquisition of isotropic voxels, which facilitate reconstruction of the coronary arteries in arbitrary and exhaustive views,<sup>64</sup> may compensate for the problem of tortuosity.

MRI characterization of human coronary atherosclerotic plaque components has been demonstrated ex vivo.<sup>65</sup> Although not yet accomplished, there is well-founded optimism<sup>63</sup> that in vivo human coronary plaque characterization will be attainable relatively soon.

## Plaque and Thrombus Characterization With Use of Contrast Agents

### Plaque Characterization

The characterization techniques described above use the inherent relaxation properties of different plaque components. Despite the use of multispectral MR<sup>58</sup> or of high (200- $\mu\text{m}^3$ ) resolution in 3D imaging,<sup>66</sup> it has still not been possible to identify uniquely plaque components. An overlap of signal intensities occurs, particularly between the lipid core and vessel media.<sup>58,66</sup> Moreover, approaches that are directed at the identification of the lipid core and fibrous cap are focused on relatively advanced lesions. More subtle distinctions within plaque and preatheromatous artery may be detectable by the introduction of paramagnetic contrast agents, such as gadolinium.

**TABLE 2. Specific Contrast Agents Used in Selected Cited Studies**

Species	Setting	Target	Contrast Agent	Contrast Weighting	Magnet, T	Reference
Human	Aorto-iliac plaque	Neovasculature	MS-325 (Gd)	$\uparrow$ (T1W)	1.5	Maki 2001 <sup>75</sup>
Human	Carotid plaque	Neovasculature	Omniscan (Gd)	$\uparrow$ (T1W)	1.5	Yuan 2001 <sup>73</sup>
Rabbit	Aortic plaque	Macrophages	SPIO	$\downarrow$ (T2W)	1.5	Schmitz 2001 <sup>79</sup>
Rabbit	Aortic plaque	Macrophages	USPIO	$\downarrow$ (T1W)	1.5	Ruehm 2001 <sup>78</sup>
Pig	Venous thrombus	$\alpha_{\text{IIb}}\beta_3$ integrin	RGD-USPIO	$\uparrow$ (T1W)	1.5	Johansson 2001 <sup>91</sup>
Ex-vivo	Thrombus	Fibrin	ACPL (Gd)	$\downarrow$ (T1/2W)	4.7	Yu 2000 <sup>88</sup>
Rabbit	Tumor angiogenesis	$\alpha_v\beta_3$ integrin	ACPL (Gd)	$\uparrow$ (T1W)	1.5	Sipkins 1998 <sup>138</sup>
Rabbit	Corneal angiogenesis	$\alpha_v\beta_3$ integrin	ACPL (Gd)	$\uparrow$ (T1W)	4.7	Anderson 2000 <sup>139</sup>
Mouse	Tumor	Transferrin receptor (transgenic)	Tf-MION	$\downarrow$ (T1/2W)	7.1	Weissleder 2000 <sup>132</sup>
Mouse	Encephalitis	ICAM-1	ACPL (Gd)	$\uparrow$ (T1W)	9.4	Sipkins 2000 <sup>137</sup>
Xenopus	Embryonic gene expression	$\beta$ -galactosidase activity	E-gadMe	$\uparrow$ (T1W)	9.4	Louie 2000 <sup>133</sup>

ACPL indicates antibody-conjugated paramagnetic liposome; E-GadMe, (1-(2- $\beta$ -galactopyranosyloxy)propyl)-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane)gadolinium(III); Gd, gadolinium-based contrast agent; ICAM-1, intercellular adhesion molecule 1; Tf-MION, human transferrin receptor–low-molecular weight-dextran–monocrystalline iron oxide nanoparticle complex; RGD-SPIO, arginine-glycine-aspartic acid peptide-SPIO conjugate; (U)SPIO, (ultra-small) superparamagnetic particles of iron oxide.

Gadolinium chelates enhance T1 relaxation and, therefore, increase contrast enhancement on T1W pulse sequences with short repetition times and echo times.<sup>67</sup> For the purposes of MR angiography, gadolinium has been used to improve blood-tissue contrast,<sup>68</sup> but it can potentially enhance the contrast in any tissue in which it resides. New microvessels form in atherosclerotic plaque, and these may be associated with features of inflammation, such as upregulation of adhesion molecules and leukocyte infiltration.<sup>69</sup> The presence of new vessels has also been associated with carotid plaque instability.<sup>70</sup> These vessels may also be abnormally permeable, allowing the extravasation of plasma proteins, such as albumin and fibrinogen.<sup>71,72</sup> In recent reports, contrast MRI has used these features to aid plaque characterization. On T1W images of carotid arteries, a gadolinium-based contrast agent has been reported to differentially enhance areas rich in plaque microvascularization and may offer a further means of distinguishing the necrotic core and fibrous cap and of highlighting at-risk plaque.<sup>73</sup> By use of MS-325, a gadolinium-based contrast agent that binds albumin, areas of high signal intensity, comparable to highly vascular tissue such as liver, have been observed in the aortic or iliac arterial wall. It has been speculated that this reflects not only increased plaque vascularity<sup>74</sup> but also a leakiness of these microvessels, which suggests active inflammation.<sup>75</sup> This is consistent with a recent report in which increased wall thickness, T2W signal, and/or gadolinium contrast enhancement in carotid arteries and aorta was associated with elevated serum levels of the inflammatory markers interleukin-6, C-reactive protein, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1.<sup>76</sup>

Contrast agents that specifically identify components of vulnerable plaque are of considerable interest. Macrophage-rich areas are a pathological correlate of unstable plaque.<sup>42</sup> Superparamagnetic nanoparticles of iron oxide (SPIO) alter the MRI reaction times and are taken up avidly by macrophages. In recent small studies, injection of SPIO into hyperlipidemic rabbits was associated with accumulation in macrophages and, after 2 hours<sup>77</sup> to 5 days,<sup>78</sup> the appearance of signal voids studded on the luminal surface of the aorta. Similar appearances have been observed incidentally in the aorta and intrapelvic arteries of humans that have received SPIO for oncological imaging.<sup>79</sup> This type of specific cellular targeting approach warrants further investigation.

### Thrombus Characterization

Plaque rupture or erosion exposes the prothrombotic core to circulating blood,<sup>10,21</sup> which can lead to acute vessel occlusion and myocardial infarction, unstable angina, or death. Recent evidence suggests that layering and organization of the thrombus may be responsible for plaque progression.<sup>23</sup> Johnstone et al<sup>80</sup> have identified the location and size of plaque-associated mural thrombus in vivo in an atherosclerotic rabbit model.<sup>80</sup> Rapid noninvasive identification and age characterization of the thrombus may be clinically useful (eg, if treatment risk versus benefit is related to the timing and location of a thrombotic event). Time-related changes in the water-diffusion properties of the thrombus have been identified by using pulse-field gradient methods.<sup>81</sup> MR signal

intensities of hemorrhage and “altered blood” depend on the structure of hemoglobin and its oxidation state.<sup>82</sup> For example, the generation of methemoglobin within an evolving thrombus is known to cause T1 shortening. This phenomenon has been exploited for the detection of fresh thrombus in the setting of deep vein thrombosis,<sup>83,84</sup> pulmonary embolus,<sup>85</sup> and acute carotid thrombus.<sup>74</sup> In these studies, direct imaging of the thrombus against a suppressed background with the use of a 3D magnetization-prepared rapid gradient echo<sup>86</sup> has been found to be effective in the imaging of thrombi.

The potential of MRI to detect arterial thrombotic obstruction and define thrombus age has been very recently evaluated by using black-blood T1W and T2W.<sup>87</sup> Carotid thrombi were induced in swine by arterial injury. Serial high-resolution in vivo MR images were obtained at 6 hours, at 1 day, and at 1, 2, 3, 6, and 9 weeks. Thrombus appearance and relative signal intensity revealed characteristic temporal changes in the MR images, reflecting histological changes in the composition. Age definition using visual appearance was highly accurate (Pearson  $\chi^2$  with 4 *df* ranging from 96 to 132 and Cohen  $\kappa$  0.81 to 0.94).

Contrast agents that characterize thrombi are under development: fibrin can be identified by lipid-encapsulated perfluorocarbon paramagnetic nanoparticles in vitro<sup>88,89</sup> and in vivo<sup>89</sup> or by a paramagnetic dendrimeric contrast agent,<sup>90</sup> whereas activated platelets can be targeted via the interaction of an ultrasmall SPIO-arginine-glycine-aspartic acid (RGD) peptide construct with the  $\alpha_{IIb}\beta_3$  receptor (Table 2).<sup>91</sup>

### Effects of Treatment

Direct plaque imaging is of potential use not only for diagnosis but also for monitoring response to treatment. Angiographic studies of progression and regression of atherosclerosis have been notoriously poor at demonstrating changes in plaque burden, even when changes in clinical event rates have been markedly altered.<sup>92,93</sup> In a study of diet/injury-induced atherosclerosis in rabbits, T2W MRI identified regression of atherosclerosis 12 to 20 months after the withdrawal of the atherogenic diet (regression group). In contrast, lesion progression was documented in rabbits that continued the atherogenic diet (progression group).<sup>94</sup> Morphometric data were presented as changes in wall thickness and percent stenosis (separate values for wall area and lumen area were not given in the study). In a similar study,<sup>95</sup> serial MRI showed a significant reduction in the lipid components of the plaque in the regression group and an increase in the progression group.

In a preliminary analysis, using PDW and T2W MRI, Corti et al<sup>35</sup> illustrated a decrease in cross-sectional wall area in atherosclerotic segments of human aorta and carotid artery (by 8% and 15%, respectively) 12 months after the initiation of simvastatin. Importantly, there was no change in cross-sectional area of the arterial lumen. This emphasizes the importance of imaging the vessel wall directly and probably explains the limitations of coronary angiography in assessing response to treatment. In another recent case-control study, 8 patients with coronary artery disease who were subjected to prolonged intensive lipid-lowering (niacin, lovastatin, and colestipol over 10 years) showed a dramatic reduction

(0.7 mm<sup>2</sup> versus 10.9 mm<sup>2</sup>,  $P < 0.001$ ) in plaque lipid content.<sup>96</sup> In that small study, differences between the groups in overall plaque area and lumen area did not reach statistical significance.

### Image Analysis

As demonstrated above, a significant strength of MRI is the ability, noninvasively, to follow the progress of lesions in individual patients over a period of time. Comparisons of this nature will provide insight into the natural history of plaque and prospective information about plaque at risk of precipitating an acute atherothrombotic event, and they will also provide information regarding response to treatment. However, changes in plaque size and composition within individuals may be small.<sup>35,96</sup> Reliable ways to ensure anatomic alignment of sections between successive scans and to measure small changes in measured parameters are required.

In our lipid-lowering study of human aortic and carotid plaques,<sup>35</sup> the reproducibility of the vessel wall area measurement was assessed after repeated imaging. The error in vessel wall area measurement was found to be 2.6% for aortic plaque and 3.5% for carotid plaque. Similar low measurement errors in plaque area and volume (4% to 6%) have been reported by others, proving that plaque area and volume can be accurately assessed.<sup>97,98</sup>

To improve quantification, semiautomatic image-processing techniques have been developed that improve the accuracy of vessel wall area measurements compared with the accuracy provided by manual morphometric analysis.<sup>99,100</sup> In one such model, a “discrete dynamic contour” is produced by image-derived edge characteristics moderated by elements to introduce contour tension and damping. Three-dimensional interpolation of discrete dynamic contours attained for inner and outer vessel walls has allowed construction of vessel wall volume,<sup>101</sup> with the potential to quantify atherosclerotic plaque burden and distribution.

### Vascular Intervention

High-resolution images of vessel wall, excellent delineation of perivascular soft tissue structures, inherent versatility of multiple plane viewing, virtual real-time images, and the ability to acquire angiographic and hemodynamic data make MRI an exceptionally promising platform for intravascular intervention.<sup>102</sup>

Preliminary studies have indicated the feasibility of MR-guided percutaneous angioplasty in rabbit aorta,<sup>103</sup> of stent deployment in pig femoral arteries,<sup>104</sup> rabbit aortas,<sup>105</sup> and pig coronary arteries,<sup>106</sup> and of the monitoring of catheter-based gene therapy in pig femoral arteries.<sup>107</sup> In humans, intraoperative MR has been shown to be safe and effective for intracranial neurosurgery,<sup>108</sup> although transvascular applications are, thus far, limited.<sup>109</sup> MR-guided coronary intervention is a relatively distant but attainable objective.

### Emerging MRI Applications and Molecular Mechanisms

#### MRI in Transgenic Mouse Models of Atherosclerosis

Mouse models have largely superseded larger animal models of human atherosclerosis (Figure 4).<sup>110–113</sup> Mice have the

advantages of small size, ample progeny, and a short reproductive cycle. Genetically modified mice spontaneously and reproducibly develop atherosclerosis that resembles that found in humans.<sup>114,115</sup> In addition, characterization of the mouse genome has enabled the application of gene knockout and transgenic technologies to study the progression<sup>116–119</sup> and regression<sup>120–123</sup> of atherosclerosis. Despite these advantages, a significant drawback of the study of such small animals has been the inability to track the progression or regression of atherosclerosis *in vivo*.<sup>124</sup>

We have previously demonstrated that MR can accurately quantify atherosclerosis in apoE-deficient mice.<sup>27</sup> By imaging at 9.4 T, with an in-plane resolution of 100×100 μm, the progression of atherosclerosis can be identified in individual mice,<sup>125</sup> and the progression of atherosclerosis can be shown to be accompanied by positive arterial remodeling.<sup>126</sup>

In these studies, atherosclerosis was quantified in the abdominal aorta. Carotid artery imaging after wire injury has also been performed in mice.<sup>127</sup> Respiratory and cardiac gating and continuous anesthetic administration will allow extended imaging to improve image quality<sup>128,129</sup> and will enable imaging of the thoracic aorta and aortic root. The aortic root is an attractive location for imaging because lesions develop earlier there than in the abdominal aorta. Furthermore, pathological studies of atherosclerosis in mice have been largely standardized to examine the aortic root.<sup>116,130</sup>

#### MRI and Molecular Imaging

The ability to image the presence or activity of specific molecules *in vivo* (Table 2) would be of considerable interest.<sup>131</sup> MRI can achieve spatial resolution to ≈10 μm. This capability exceeds that of positron emission tomography, single positron emission CT, and nuclear techniques.

Weissleder et al<sup>132</sup> have refined and extended the application of superparamagnetic contrast through transgenic expression of transferrin receptor in nude mice. The resultant increase of the uptake of injected superparamagnetic iron nanoparticles significantly decreased the MR signal in a tumor model, relative to transferrin receptor-negative controls, such that the expression of the transgene could be mapped noninvasively by the use of MRI.

Louie et al<sup>133</sup> have developed an MR contrast agent capable of reporting the activity of a β-galactosidase. The paramagnetic agent (abbreviated EgadMe) requires interaction with a water molecule to generate an increased MR signal in T1W images. In the resting state, however, its interaction with water is prevented by the attachment of galactopyranose, a “blocking group” that is susceptible to enzymatic cleavage by β-galactosidase. The subsequent association of water with EgadMe results in an increase of T1 signal by ≈60%. By use of this approach, it was possible to localize the lineage of a cell injected with β-galactosidase mRNA in early embryonic development. The ability to image areas in which β-galactosidase is active may be of considerable use in the study of transgenic animals and in mapping sites of expression *in vivo* in gene therapy. Moreover, this technique may represent a paradigm of intelligent contrast agents that are activated in response to specific biological

events. EganMe and its successors may be conjugated with blocking units that are substrates for other enzymes. In the context of atherosclerosis, matrix metalloproteinases (MMPs) digest collagen, elastin, and other matrix components. Some MMPs are believed to be important in the generation of unstable plaque<sup>20</sup>; thus, identification of vulnerable plaque may be feasible by targeting specific proteolytic activities. MMPs have the additional advantage of extracellular activity, thus circumventing the problem of intracellular access by contrast agents.

The endothelial cell surface proteins, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, are upregulated in atherosclerotic plaque<sup>134,135</sup> and in areas of artery prone to lesion formation.<sup>136</sup> Exposure to circulating blood renders such molecules potential targets for monoclonal antibody-conjugated intravascular MR contrast agents. Antibodies conjugated to paramagnetic liposomes have been used to image, *ex vivo*, intercellular adhesion molecule expression in a murine model of multiple sclerosis<sup>137</sup> and  $\alpha_v\beta_3$  integrin expression as a marker of angiogenesis.<sup>138,139</sup> Where cells are accessible to blood, perhaps as a consequence of abnormal vascular permeability in plaques, imaging specific receptor expression with the use of contrast-ligand constructs should also be feasible.<sup>140</sup>

### Conclusions

In the future, clinical investigation of atherosclerosis will not be restricted by the endoluminal approach that has limited x-ray contrast arteriography. High-resolution noninvasive MRI will provide exhaustive 3D anatomic information about the lumen and the vessel wall. Furthermore, MRI has the ability to characterize plaque composition and microanatomy and, therefore, to identify lesions vulnerable to rupture or erosion. This may aid in early intervention in the primary and secondary treatment of vascular disease. The high resolution of MRI and the development of sophisticated contrast agents offer the promise of molecular *in vivo* molecular imaging of the plaque.

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