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Utilizing imaging tools in lipidology: examining the potential of MRI for monitoring cholesterol therapy

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Abstract

Lipid abnormalities play important roles in the development of atherosclerosis. Lipid therapies result in alterations in atherosclerotic plaques including halting of progression of the plaque, lipid transport out of the plaque and reducing inflammatory activity, which lead to plaque morphologies that are less prone to disruption, the main cause of clinical events. In order to investigate and monitor plaque morphological changes during lipid therapy *in vivo* we need an imaging method that can provide accurate assessment of plaque tissue components and activity. MRI of atherosclerosis has been validated as a reliable assessment of the size of the vessel lumen, but also the size of the plaque, its tissue composition and plaque activity, including inflammation. The purpose of this review is to summarize the state of evidence for the direct assessment of atherosclerotic plaque and its change by MRI, and to establish the proven role of MRI of atherosclerosis in pharmaceutical trials with lipid therapy.

Keywords

atherosclerosis; lipids; MRI

Atherosclerotic disease remains the leading cause of death and disability in the USA and Europe [1,2]. Lipid abnormalities play important roles in the development of atherosclerosis [3].

The link between therapeutic alterations in serum lipids and reduced risk from atherosclerosis arises from alterations in atherosclerotic plaques in response to therapy. These responses may include halting of progression of the plaque, lipid transport out of the plaque and reduction of inflammatory activity within the plaque. These latter responses are especially important, as they lead to plaque morphologies that are less prone to disruption, the proximate cause of clinical events [4–9].

Evaluation of effects of lipid therapy on atherosclerotic plaques should be focused on plaque tissue composition and inflammatory activity in addition to plaque burden assessment. Several imaging methods, including x-ray angiography [10,11], ultrasound [12,13] and computed tomography [14] have been reported for assessing the severity of atherosclerosis

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and studying arterial walls. However, none of these imaging methods can completely characterize the composition of plaques [15].

Development of high-resolution MRI techniques in recent years has made direct assessment of plaque tissue composition possible. Numerous studies have shown that MRI exhibits high contrast for internal plaque features, and that combined information from multiple contrast weightings is critical for distinguishing all plaque components [16–21]. With confirmation from histological studies, high-resolution multi-contrast MRI can accurately assess plaque tissue contents [22–24]. The purpose of this review is to summarize the current state of MRI of atherosclerosis and to discuss the potential utility of MRI in monitoring lipid therapy. Because the carotid artery has been the major focus of research and development in MRI of atherosclerosis, this review will mainly cover MRI of carotid atherosclerosis.

Roles of lipids in atherosclerotic plaque formation

Lipid abnormalities play a critical role in the development of atherosclerosis [3]. Early experiments in animals demonstrated accelerated atherosclerosis with a high-cholesterol diet. This was followed by epidemiologic studies conducted in countries around the world that showed an increasing incidence of atherosclerosis when serum cholesterol concentrations were elevated. The roles of each LDL, HDL, metabolic dyslipidemia and inflammation is discussed in detail below.

LDL

Endothelial dysfunction induced by risk factors including dyslipidemia, such as oxidized LDL, an initial step in atherosclerosis. In patients with hypercholesterolemia, LDL particles infiltrate the artery in excess and are retained in the intima, particularly at sites of hemodynamic strain [25]. As demonstrated in Figure 1, oxidative modification of LDL is a prerequisite for macrophage uptake via unregulated macrophage scavenger receptors (CD36, also known as scavenger receptor B, appears to be the most important) and accelerated accumulation of cholesterol [26,27]. Macrophage uptake of LDL-cholesterol may initially be an adaptive response, which prevents LDL-induced endothelial injury [28]. However, cholesterol accumulation in foam cells leads to mitochondrial dysfunction, apoptosis and necrosis, with a resultant release of cellular proteases, inflammatory cytokines and prothrombotic molecules [28].

LDL is known to comprise multiple subclasses differing in size and density and in their relationships with cardiovascular disease risk [29–31]. It has been suggested that small, dense LDL particles have a greater arterial proteoglycan binding and oxidative susceptibility, which may be directly responsible for their enhanced atherogenic potential; however, there is insufficient evidence to assess the extent to which increased levels of these particles denote other pathologic mechanisms [30,31].

HDL

By contrast to LDL, HDL has antiatherogenic properties that include reverse cholesterol transport, maintenance of endothelial function, protection against thrombosis and maintenance of low blood viscosity through a permissive action on red cell deformability. The net effect is that there is an inverse relationship between plasma HDL-cholesterol (HDL-C) levels and cardiovascular risk [32]. HDL's antiatherosclerotic effect is attributed in part to its ability to mobilize excess cholesterol from artery wall macrophages [33,34]. Two pathways involve membrane ATP-binding cassette transporters (ABCA1 and ABCG1), which are highly induced when macrophages over accumulate cholesterol [33–39]. ABCA1 mediates the transport of cholesterol and phospholipids from cells to lipid-poor apolipoproteins [33,34]. ABCG1 mediates the transport of cellular cholesterol to HDL

particles [35–39]. HDL-mediated reverse cholesterol transport from arterial wall macrophages has become an important target for a better understanding of atherosclerosis process and development of new antiatherosclerosis therapy [40].

The static measurement of HDL-C levels has inherent limitations as a metric of the functional effects of HDL *in vivo*. Reports of marked heterogeneity in the particle composition and biologic properties of HDL have reinforced a need for validated assays of HDL function [41]. Rader and colleagues tested the hypothesis that cholesterol efflux capacity is a determinant of atherosclerotic burden that is independent of the HDL-C level [42]. They found that the levels of HDL-C and ApoA-I were significant determinants of cholesterol efflux capacity but accounted for less than 40% of the observed variation. An inverse relationship was noted between efflux capacity and carotid intima-media thickness by B-model ultrasound both before and after adjustment for the HDL-C level. Furthermore, efflux capacity was a strong inverse predictor of coronary disease status by coronary angiography (adjusted odds ratio for coronary disease per one standard deviation increase in efflux capacity: 0.70; 95% CI: 0.59–0.83; $p < 0.001$) [42].

Metabolic dyslipidemia

Metabolic dyslipidemia consists of a low level of HDL-C together with elevated triglycerides and a preponderance of small, dense LDL particles [3]. LDL-cholesterol may be only minimally elevated. This phenotypic pattern is characteristic of patients with diabetes or metabolic syndrome [3], the presence of each of which significantly increases the risk for cardiovascular disease [43,44]. Not surprisingly, the metabolic dyslipidemia with multiple lipid abnormalities attributes further increased risk for atherosclerosis progression and clinical events. This phenomenon was confirmed by a combined analysis, examining the impact of metabolic syndrome on coronary stenosis progression and major cardiovascular events. Zhao *et al.* found that patients with metabolic syndrome had 50% more rapid coronary stenosis progression and a 64% increased cardiovascular event rate [45].

Inflammation

Atherosclerosis is considered an inflammatory disease because macrophages that have been modified by oxidized LDL release a variety of inflammatory substances, cytokines and growth factors [46]. Among the many molecules that have been implicated are: MCP-1 [47,48]; ICAM-1 [48]; GM-CSFs [49,50]; soluble CD40 ligand [51]; IL-1, IL-3, IL-8 and IL-18 [51–54]; and TNF- α [55–57]. The inflammatory process is noted in all stages in the development of atherosclerosis [58,59].

Specifically related to plaque stability, macrophages within the plaques are capable of degrading the extracellular matrix by secreting enzymes, such as plasminogen activators and matrix metalloproteinases (collagenase, gelatinase and stromelysins) that weaken the fibrous cap, making plaques more prone to rupture [58,59]. Tissue factor produced by macrophages in plaques is found to be a prothrombotic trigger [60]. van der Wal *et al.* demonstrated that an abundant concentration of inflammatory cells, including macrophages and T lymphocytes, was often found at the immediate site of intimal rupture or erosion of thrombosed coronary arteries in patients who died of acute myocardial infarction [8]. Macrophage rich areas were more commonly found in atherosclerotic plaques in patients with unstable angina compared with patients with stable angina [9].

Features of advanced atherosclerotic plaques

Atherosclerosis alone is thought to be a relatively benign disease and progresses with aging; however, it is frequently complicated by acute thrombosis, usually triggered by the rupture or erosion of an atherosclerotic plaque [61,62]. Advanced lesions at risk for rupture

generally share one or more common morphologies. The high-risk plaque features, including a thin-cap fibroatheroma, a lipid-rich core, intraplaque hemorrhage (IPH) and neovascularization are described in this section.

Thin-cap fibroatheroma

During the progression of atherosclerosis, endothelial cells, macrophages and smooth muscle cells die from apoptosis or necrosis [63]. Disintegration of foam cells, loss of smooth muscle cells and the production of matrix metalloproteinases by activated leukocytes have detrimental consequences – leading to the formation of a destabilizing lipid-rich necrotic core (LRNC) and a potentially fragile and rupture-prone fibrous cap (Figure 2) [64,65]. Plaques at risk of rupture are often composed of a large lipid core, a thin fibrous cap and shoulder regions with a high density of inflammatory cells and microvessels [7–9,58–64]. This type of lesion is referred to as thin-cap fibroatheroma. According to the pathology reports, two-thirds of acute coronary events result from the rupture of a thin-cap fibroatheroma; the remaining events are caused by erosion of the intimal surface with subsequent local thrombus formation.

Pathological studies of the carotid artery report similar findings relating carotid plaque features to acute cerebrovascular events [66]. However, eroded lesions were found to be rare in the carotid arteries. On the other hand, ulcerated plaques, exhibiting excavations where a portion of the plaque has embolized, were found to be common in the carotid arteries.

Intraplaque hemorrhage

Carotid arteries and, to a lesser extent, coronary arteries also frequently exhibit IPH, thought to arise from immature neovessels within the plaque [67]. IPH is another route for lipid accumulation in the plaque, derived from the cell walls of red blood cells [68]. Indeed, carotid artery plaques exhibiting IPH have been found to progress more rapidly in both wall area and LRNC size [69]. Presence of IPH has also emerged as a potential risk factor for stroke in several prospective studies of carotid atherosclerosis [70–72].

Neovascularization

There is evidence that hypoxia plays an important role in triggering microvessel proliferation in the inner layers of the vessel wall, to compensate for the limited diffusion of oxygen in large atherosclerotic plaques. Neovascularization appears to be another important step in the progression of atherosclerotic plaques [73–75]. The new microvessels originate from adventitial vasa vasorum. They are immature, fragile and leaky and express cellular adhesion molecules, resulting in local extravasation of plasma proteins, erythrocytes (bleeding) and inflammatory cells, which are thought to be the mechanisms for further macrophage accumulation and IPH [70]. Moreno *et al.* discovered that the density of plaque microvessels was increased in ruptured aortic atherosclerotic plaques and correlated with the degree of macrophage infiltration, often found at the shoulder of plaques [9].

MRI of atherosclerotic plaques

The association of several morphological features with atherosclerotic plaque vulnerability has naturally led to efforts to measure these features by noninvasive imaging. In order to study plaque morphology *in vivo* we need an imaging method that can provide an accurate assessment of plaque tissue components and activity. As mentioned in the introduction, several imaging methods, including x-ray angiography [10,11], ultrasound [12,13] and computed tomography [14] have been reported for assessing the severity of atherosclerosis and studying arterial walls. However, none of these imaging methods can completely characterize the composition of plaques [15]. Development of high-resolution MRI

techniques in recent years has made direct assessment of plaque tissue composition possible using multiple contrast-weighted protocols (Figure 3). The capability of MRI of plaque burden, LRNC and other tissue components, IPH, and neovascularization and inflammation are described in this section.

Plaque burden

The first obvious target for MRI of atherosclerosis is to measure the size of the artery wall. While magnetic resonance angiography is widely available and can provide bright lumen signals suitable for measuring stenosis and identifying ulcerations in the lumen surface [76], MR A is unable to depict plaque burden. For this purpose, multiple-contrast black-blood imaging techniques have been employed for cross sectional imaging of the artery wall [77]. Luo *et al.* compared *in vivo* measurements of 33 carotid arteries obtained with a combination of T1-weighted (T1W), T2-weighted (T2W) and proton-density-weighted MRI to corresponding *ex vivo* measurements after endarterectomy [78]. Measurements were highly correlated (coefficients in excess of 0.9) and differed by an amount consistent with the thin rim of the artery left *in vivo* after surgery.

Lipid-rich necrotic core

Such multiple contrast techniques have also been widely utilized for the detection and measurement of plaque components, including LRNC. According to Saam *et al.*, the LRNC is characterized by an isointense to hyperintense signal shown on T1W images and hypointense signal on T2W images (Table 1) [79]. Using these criteria, a correlation of 0.75 was found between MRI and histological measurements of the LRNC area. Further improvements have been made using gadolinium-based contrast agents in conjunction with T1W imaging, which yield a higher contrast-to-noise ratio for the LRNC than T2W images [80]. Using this approach, Cai *et al.* reported a correlation of 0.84 between MRI and histological measurements of LRNC size (Figure 4) [24]. This feature was also employed by Takaya *et al.* to show that the addition of contrast-enhanced T1W images to the protocol reduced the inter-rater coefficient of variation for measuring LRNC size from 33.5 to 17.6% [23].

IPH & other plaque components

In addition to providing criteria for the identification of LRNC, Saam *et al.* provided guidelines for identifying calcification, IPH and loose fibrous matrix based on relative signal intensities in each of the contrast weightings, as shown in Table 1 [79]. In 214 matched cross-sectional locations, the measured areas of these components correlated with corresponding histological measurements with coefficients in excess of 0.7. Robust signal characteristics have also enabled the development of automated classifiers that identify these regions with accuracies comparable to expert human readers [81].

The ability of these techniques to identify IPH is one of the most compelling reasons for the use of MRI in atherosclerosis imaging. Methemoglobin in IPH serves as an endogenous contrast agent, leading to shortened longitudinal relaxation constant T1 and hyperintensity on T1W images (Figure 5). This hyperintense property of IPH has been noted on general-purpose T1W images, including spin echo and time-of-flight acquisitions [82,83]. A heavily T1W sequence using inversion recovery magnetization prepared rapid gradient echo has also been proposed [84]. In a comparison of magnetization prepared rapid gradient echo with general-purpose T1W sequences, Ota *et al.* found that magnetization prepared rapid gradient echo had the best overall diagnostic accuracy compared with histology, achieving a sensitivity of 80% and specificity of 97% [85]. Further improvements have been proposed by Wang *et al.* by using phase-sensitive reconstruction to achieve higher IPH contrast and

improved lumen differentiation [86]. MRI is also able to differentiate between recent and older IPH using T2W signal characteristics [82].

Plaque neovasculature & inflammation

Dynamic contrast-enhanced (DCE)-MRI after administration of gadolinium agents can be used to assess plaque perfusion arising from the adventitial vasa vasorum (Figure 6). Kinetic modeling of DCE-MRI is firmly established in oncology, where elevated parameters indicative of vascularity and permeability are associated with malignant tumors [87]. The fact that enhanced blood supply and permeability are also associated with inflammation has led researchers to investigate the ability of DCE-MRI to detect inflammation [88,89] and to investigate its use for characterizing plaque inflammation. In a study of 16 subjects undergoing carotid endarterectomy, Kerwin *et al.* reported a correlation of 0.8 between estimates of fractional blood volume estimated by kinetic modeling of DCE-MRI and histological measurements of neovessel area [90]. In a follow-up study, both fractional blood volume and the transfer constant, which is sensitive to vessel permeability, were found to correlate with plaque macrophage content [91]. Macrophage content has been directly targeted using injected ultrasmall particles of iron oxides, which are phagocytosed by macrophages and lead to focal signal losses peaking 24–36 h after injection [92].

Association of plaque characteristics by MRI with lipid levels & therapy

Although MRI of atherosclerosis has primarily been motivated by the need for better diagnostic risk assessments, its development has had the auxiliary benefit of providing a powerful tool for understanding the natural history of the disease. In particular, MRI permits the evaluation of the link between clinical risk factors and the development of vulnerable plaque.

Plaque features & HDL-C

An inverse relationship exists between reduced levels of HDL-C and an increased risk of cardiovascular disease [32], which is thought to arise from facilitation of cholesterol removal by HDL. MRI studies support this antiatherogenic property of HDL. Reports demonstrate a significant inverse association between plaque LRNC measured *in vivo* by MRI and serum HDL-C ($r = -0.68$; level ($r = -0.5$; $p = 0.01$) and HDL3 ($p = 0.02$), and an inverse association between carotid wall volume and HDL-C ($r = -0.39$, $p = 0.02$) [93,94]. HDL is also anti-inflammatory, which may explain a reported inverse relationship between the DCE-MRI marker the transfer constant and HDL-C [91,95].

Plaque features & lipid therapy

The most widely accepted explanation for the clinical event reduction with statin therapy is that lipid-lowering targets the plaque rupture risk features such as a large lipid core, a thin fibrous cap and a high level of inflammatory infiltrates and activity; therefore, the plaque stability is improved and cardiovascular events are reduced. The role of lipid therapies in yielding these differences in plaque morphology has been investigated in several case-control and cross-sectional studies. In a study by Zhao *et al.*, the carotid arteries of eight subjects with coronary artery disease undergoing long-term (10-year) lipid-lowering therapy were compared with those of eight matched controls who had never been treated with lipid-lowering therapy [96]. Although the treated subjects exhibited plaques that were only marginally smaller in area (58 [treated] vs 64 mm² [control], $p =$ not significant), their lesions had significantly smaller LRNC areas (0.7 vs 10.2 mm², respectively; $p = 0.01$). Studies by Saam *et al.* [97] and Underhill *et al.* [98] examined clinical factors associated with progression in plaque size over 18 months. In both studies, statin use emerged as an independent predictor of reduced rates of progression. Interestingly, in the Underhill study, a

trend emerged for more rapid progression when IPH was present in the plaque, regardless of statin use, suggesting IPH might provide a pathway for LRNC accumulation that does not respond to statins [98].

Clinical trials of plaque change in response to lipid therapy by MRI

In a more rigorous study design, MRI of atherosclerosis also serves as a logical end point in clinical trials of lipid therapies. MRI offers a major advantage in such roles because it can be performed noninvasively for serial studies in order to observe temporal changes in plaque. Furthermore, the changes are quantitative and continuous variables that provide substantial gains in statistical power, enabling studies to be performed with relatively few subjects.

Plaque volume

A study by Corti and colleagues followed aortic and carotid plaques in hypercholesterolemic patients under simvastatin therapy for 2 years, with MRI at 6-month intervals [99,100]. The vessel wall area was reduced by 11% after only 1 year ($p < 0.01$), with more moderate increases in lumen area reaching significance by 18 months.

MRI was also used in a dose-finding study of ETC-216, a recombinant ApoA-I Milano/1-palmitoyl-2-oleoyl phosphatidyl-choline complex, on injury-induced carotid atherosclerotic plaques in cholesterol-fed New Zealand white rabbits [101]. The study demonstrated that there was significant plaque regression, approximately 6% as assessed by both MRI and intravascular ultrasound, in ETC-216-treated rabbits compared with placebo.

The recent development of CETP inhibition has attracted much attention. CETP is a plasma protein that mediates the exchange of cholesteryl ester in HDL for triglyceride in VLDL [102,103]. This process decreases the level of antiatherogenic HDL-C and increases proatherogenic VLDL and LDL-cholesterol; therefore, CETP is potentially atherogenic [104–107]. On the other hand, CETP could also be antiatherogenic [108–111], because it participates in reverse cholesterol transport (the transfer of cholesterol from peripheral cells through the plasma to the liver) [112]. Despite the ongoing debate over the role of CETP in atherosclerosis, a recent study of dalcetrapib in 130 subjects with or at high risk of coronary heart disease showed that total vessel area was reduced in patients given dalcetrapib at 600 mg/day compared with those given a placebo after 24 months; the absolute change from baseline relative to placebo was -4.01 mm^2 (90% CI: -7.23 to -0.80 ; nominal $p = 0.04$) [113].

Plaque lipid depletion during lipid-lowering therapy

Atherosclerosis regression was first studied in animal models in 1970s and 1980s. Several studies in nonhuman primates showed that normalizing and lowering cholesterol have led to a disappearance of lipid-laden macrophages (foam cells) within 6 months; core lipid volume begins to diminish after 6 months; after 2 years, 60% of plaque cholesterol is depleted and plaque shrinkage is primarily due to cholesteryl ester depletion [114–117]. This was confirmed in human histological observations. In a case-control study of patients randomized to 3 months of pravastatin treatment or placebo prior to carotid endarterectomy, Crisby *et al.* provided histological evidence of the efficacy of therapy on plaque lipid content [118].

The Carotid Plaque Composition by MRI during Lipid Therapy (CPC) study was designed as a randomized, double-blind, partially placebo-controlled study in which patients with known coronary or carotid disease were randomized to one of three intensive lipid regimens, all of which included atorvastatin [119]. Annual serial MRI data from 33 pooled patients with a LRNC on baseline MRI showed a 3-year reduction in average percentage LRNC

from 14.2 to 7.4% ($p < 0.001$), with a significant 1-year reduction over each of the first and second years, but a small nonsignificant decrease in year 3 [120]. An example of plaque lipid depletion and its time course are shown in Figure 7. The CPC study also revealed a reduction in vessel wall volume that became significant at 2 years. Notably, the CPC study demonstrated that the regression observed in plaque burden was primarily induced by lipid depletion, because substantial and statistically significant carotid wall burden reduction only occurred in the slices containing a LRNC as shown in Table 2 and the time course of wall burden reduction seemed to follow plaque lipid depletion, with the largest changes occurring during the second year. In another study to investigate the effect of rosuvastatin on LRNC size, Underhill *et al.* reported a 41.4% reduction in the proportion of the vessel wall consisting of LRNC after 2 years in 33 subjects ($p = 0.005$) [121]. No significant change in plaque volume was observed.

Plaque inflammation & neovascularization reduction with lipidlowering therapy

Pharmaceutical studies have also been undertaken using contrast agents to assess the response of inflammatory factors in atherosclerotic plaques. In the ATHEROMA study, Tang *et al.* reported a significant ($p = 0.0003$) reduction in the uptake of ultrasmall particles of iron oxides after only 6 weeks on 80 mg atorvastatin [122]. No change was observed in the 10-mg group. In the CPC study described previously, DCE-MRI was used to investigate changes in vasa vasorum under lipid therapy. Dong *et al.* reported a drop in the transfer constant from 0.085 to 0.067 min^{-1} ($p = 0.02$) [123].

Future perspective of monitoring lipid therapy using MRI

MRI in multiple artery beds

Although the overwhelming majority of imaging development has been based on carotid MRI, similar techniques have been applied to the aorta, coronary arteries, intracranial arteries and peripheral vasculature of the legs. Each of these targets introduces unique challenges including small vessel size (intracranial and coronary arteries), deep location and motion (coronary arteries and aorta), highly distributed lesions (peripheral arteries) and tortuosity (intracranial arteries and the aortic arch). To address these challenges, emerging 3D and rapid imaging protocols are especially promising. The use of 3D sequences leads to improved signal-to-noise ratios, increased longitudinal coverage, improved resolution especially in the through-plane direction and greater acquisition efficiency [124–126].

Molecular imaging & nanotechnology

MRI is promising for targeted molecular imaging of the specific characterization of different plaque components. In particular, HDL nanoparticles, both synthetic and reconstituted from human serum, are a flexible platform for the delivery of contrast agents to atherosclerotic plaques. A number of approaches for HDL-based contrast agents have been described that incorporate either gadolinium or iron oxide into the nanoparticles and have been shown to alter vessel wall contrast 24–72 h after administration in mouse models of atherosclerosis [127]. By also tagging the nanoparticles with fluorescent markers, *ex vivo* analyses have confirmed the specific uptake of these particles in macrophages [128]. These studies suggest the potential to use HDL contrast agents in future animal studies of lipid transport or response to therapy and, ultimately, in human studies. Nanoparticles may also be developed that incorporate therapeutic payloads for combined diagnostics and treatment.

In addition to HDL nanoparticles, a number of other targeted agents with potential applications in monitoring lipid therapy are under development. Plaque neovascularization can be imaged with $\alpha_v\beta_3$ -integrin-targeted paramagnetic nanoparticles or a mimetic of arginine-glycine-aspartic acid peptide grafted to gadolinium-diethylenetriamine penta-acetic acid

[129,130]; VCAM-1-expressing endothelial and inflammatory cells can be detected with a dedicated agent [131]. For now, all of these technologies are mainly applicable in animal studies of atherosclerosis.

Associations of biomarkers, plaques & clinical outcomes

Lipid therapies are often developed based on the expected mechanistic action to change cholesterol metabolism and, thus, impact the atherosclerosis process. Ongoing and future studies will provide the link between the changes in biomarker and plaque features, as well as the link to clinical outcomes. Establishment of plaque changes leading to different clinical outcome status will yield cost-effective strategies for atherosclerosis research.

Conclusion

MRI as a noninvasive and nonradiation technique has been extensively validated for atherosclerosis imaging. Preliminary evidence indicates that unique features of carotid plaques identified by MRI, such as IPH, LRNC and fibrous cap rupture, are associated with a higher risk of clinical and subclinical events.

In the investigation of therapeutic mechanisms, MRI of atherosclerosis is able to depict changes in plaque in trials that are much smaller than would be required to observe changes in survival. Studies to date indicate that changes in lumen size are slow to develop, whereas changes in wall area appear to be detectable earlier. Changes in LRNC appear to be observable even earlier and may provide a vascular biological explanation for the onset and persistence of clinical benefit seen in the placebo-controlled lipid-lowering trials. The cardiovascular event reduction in these trials began at 1–2 years after initiation of lipid-lowering therapy, which corresponds with the timing of significant plaque lipid depletion. Markers of inflammation may be the fastest to respond to therapy. Knowledge of such time-dependent responses of different markers can be valuable for designing clinical trials based on the expected mechanism of treatment.

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Executive summary

Lipid abnormalities play a critical role in the development of atherosclerosis

- LDL particles in excess infiltrate the artery and are retained in the intima. Macrophage uptake of modified LDL particles leads to cell accumulation and plaque formation.
- HDL is thought to have antiatherogenic properties that include reverse cholesterol transport, maintenance of endothelial function, protection against thrombosis and maintenance of low blood viscosity through a permissive action on red cell deformability.
- Metabolic dyslipidemia with multiple lipid abnormalities, including a low level of HDL-cholesterol together with elevated triglycerides and a preponderance of small, dense LDL particles, indicated a further increased risk for atherosclerosis progression and clinical events.
- Inflammation is involved in plaque formation and instability.

Features of advanced atherosclerotic plaques

- Thin-cap fibroatheroma composed of a large lipid core, thin fibrous cap and shoulder regions with a high density of inflammatory cells and microvessels is attributed to two-thirds of acute coronary events.
- Intraplaque hemorrhage, particularly in carotid arteries, is a high-risk plaque feature.
- Neovascularization appears to be another important step in the progression of atherosclerotic plaques. It is found in ruptured aortic atherosclerotic plaques and is correlated with the degree of macrophage infiltration.

MRI of atherosclerotic plaques

- MRI is a noninvasive radiation technique.
- MRI is validated as a reliable assessment of: plaque burden, lipid-rich necrotic core and other tissue components, intraplaque hemorrhage; and neovascularization and inflammation.

Association of plaque characteristics by MRI with lipid levels & therapy

- Lipid-rich necrotic core and plaque inflammation are inversely associated with low level of HDL-cholesterol.
- Lipid therapy is associated with less plaque lipid content but not with intraplaque hemorrhage.

Clinical trials of plaque change in response to lipid therapy by MRI

- Lipid therapy reduces plaque volume as assessed by MRI.
- Lipid therapy depletes plaque lipid content as assessed by MRI.
- Lipid therapy decreases plaque inflammation and neovascularization as assessed by dynamic contrast-enhanced MRI.

Future perspective of monitoring lipid therapy using MRI

- 3D sequences lead to improved signal-to-noise ratios, increased longitudinal coverage, improved resolution (especially in the through-plane direction) and greater acquisition efficiency.
- MRI is promising for targeted molecular imaging of the specific characterization of the different plaque components.
- The links between the changes in biomarker and plaque features and between plaque changes and clinical outcomes need to be established.

Figure 1. The initial events of atherogenesis

Native LDL becomes trapped in the subendocardial space and can undergo oxidation by macrophages as well as smooth muscle and endothelial cells. Oxidized LDL can (A) stimulate monocyte chemotaxis and (B) inhibit the monocyte from exiting through the vascular wall. (C) Monocytes transform into macrophages that take up oxidized LDL, becoming foam cells. (D) Oxidized LDL can also lead to foam cell necrosis with the release of lysosomal enzymes and endothelial injury and dysfunction (E).

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Figure 2. Histological features of vulnerable carotid atherosclerotic plaque
FC: Fibrous cap rupture; IPH: Intraplaque hemorrhage; NC: Necrotic core.

Figure 3. MRI example of eight consecutive images of the common carotid arteries through bifurcation to the internal carotid arteries (from 1 to 8) from five contrast weightings (time-of-flight, proton-density-weighted, T2-weighted, T1-weighted and postcontrast T1-weighted) The lumen (in red) and outer wall boundary (in blue) of the carotid artery are identified and outlined. Plaque tissue components within the arterial wall are identified using an established algorithm and quantified (loose matrix in purple and lipid content in yellow). For example, lipid content in yellow appears isointense on the TOF and precontrast T1W images, hypointense on PDW and T2W images, and is not enhanced compared with the surrounding tissue on postcontrast T1W images. Total lipid-rich necrotic core (LRNC) volume is calculated by multiplying the slice thickness (2 mm) by the sum of the areas circled in yellow on the eight consecutive images. Percentage LRNC (% LRNC) is the proportion of the wall occupied by the LRNC in only the lipid containing slices, from slice 2 to 7.

CCA: Common carotid arteries; CE: Contrast-enhanced; ICA: Internal carotid arteries; PDW: Proton-density-weighted; T1W: T1-weighted; T2W: T2-weighted; TOF: Time-of-flight.

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Figure 4. Cross-sectional appearance of lipid-rich necrotic core by common imaging techniques (A) 3D time-of-flight magnetic resonance angiography; (B) T1-weighted turbo spin echo (TSE); (C) proton-density-weighted TSE; (D) T2-weighted TSE; (E) contrast-enhanced T1-weighted TSE; (F) contrast-enhanced T1-weighted TSE with lipid-rich necrotic core indicated in yellow and enhancing fibrous cap indicated by 'a'; (G) corresponding histology with calcifications indicated by arrows; (H) high-resolution view of non-enhancing necrotic core and (I) high-resolution view of enhancing fibrous cap. Carotid artery lumen are indicated by asterisks.

H&E: Hematoxylin and eosin stain; JV: Jugular vein.

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Figure 5. Cross-sectional appearance of intraplaque hemorrhage by common imaging techniques (A) 3D TOF magnetic resonance angiography; (B) T1W turbo spin echo (TSE); (C) PDW TSE; (D) T2W TSE; and (E) corresponding histology. Asterisks indicate carotid artery lumen.

PDW: Proton-density-weighted; T1W: T1-weighted; T2W: T2-weighted; TOF: Time-of-flight.

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Figure 6. Example of vasa vasorum image and kinetic modeling

Kinetic analysis of dynamic contrast-enhanced MRI of carotid atherosclerosis yields a parametric image (A) displaying the fractional blood volume in red and K^{trans} in green. Image is derived from kinetic modeling of changing intensity in serial images (B) coinciding with contrast agent injection. Arrows indicate rim of high adventitial K^{trans} .

K^{trans} : Transfer constant.

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Figure 7. MRI example of the plaque lipid depletion and its time course

Regression in lipid-rich necrotic core size was notable between the baseline, 1-year and 2-year MRI scans. Regression in plaque volume seemed to follow plaque lipid depletion and was most pronounced from years 1–3. Arrows indicate an area of large lesion with the most pronounced regression.

CE-T1W: Contrast-enhanced T1-weighted.

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Table 1

Tissue classification criteria.

MRI sequence	LRNC with:		Calcification	Loose matrix	Dense (fibrous) tissue
	No or little hemorrhage	Recent hemorrhage			
TOF	0	+	-	0	-
T1W	o/+	+	-	-/o	0
PDW	-/o	+	-	+	0
T2W	-/o	+	-	+	0
>80% SI postcontrast T1W	-	+	-	+	+

Classification criteria are based on the following codes for signal intensity relative to adjacent muscle: +: Hyperintense; -: Hypointense; o: Isointense.

LRNC: Lipid-rich necrotic core; PDW: Proton-density-weighted; SI: Signal intensity; T1W: T1-weighted; T2W: T2-weighted; TOF: Time-of-flight.

Data taken from [79].

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Carotid wall measurement changes over 3 years and differential changes between slices with and without a lipid-rich necrotic core.

Table 2

		Comparison: baseline vs 3 years			Changes over 3 years [†]			Comparison: change in slices over 3 years LRNC (+) vs LRNC (-)				
	n	Baseline	3 years	p-value [‡]	n	1st year	2nd year	3rd year	n	LRNC (+)	LRNC (-)	p-value
Wall volume (mm ³)	33	641 ± 200	597 ± 198	<0.001	30	-16 ± 62	-42 ± 57 ^{§¶}	3 ± 34	31	-45 ± 55 [#]	-11 ± 32	0.004
Wall volume (%)	34	46.5 ± 7.1	44.3 ± 7.2	0.006	30	-0.6 ± 2.9	-2.1 ± 3.2 ^{§¶}	-0.2 ± 2.5	31	-4.7 ± 7.1 [#]	-1.4 ± 3.2	0.02
Lumen volume (mm ³)	34	750 ± 249	764 ± 258	0.11	30	2 ± 50	18 ± 52	5 ± 42	31	12 ± 41	7 ± 40	0.3

[†] Changes between the scans were annualized owing to different times between the visits for different patients.

[‡] Paired t-test.

[§] p < 0.001 when compared with baseline.

[¶] p 0.01 when compared with year 1.

[#] p < 0.001 when compared to zero change.

(-): Absence; (+): Presence; LRNC: Lipid-rich necrotic core.

Data taken from [120].