Atherosclerosis and its thrombotic complications still remain the major cause of morbidity and mortality in western societies. Atherogenesis in humans generally occurs over decades, and lesion evolution and growth may vary according to heredity, gender, lifestyle and environmental conditions. However, the development of animal models of experimental atherosclerosis and the emergence of several imaging modalities have provided indispensable knowledge to our understanding of the fundamental mechanisms of disease progression and allowed the in vivo detection of atherosclerosis in animals and humans. MRI has evolved as one of the leading noninvasive imaging modalities to visualize the vessel wall with high spatial resolution and without ionizing radiation. This article summarizes the currently available animal models of experimentally induced atherosclerosis and the application of MRI in preclinical and clinical imaging studies.

**KEYWORDS:** animal model, atherosclerosis, contrast agent, molecular imaging, MRI, thrombosis

Atherosclerosis is a progressive arterial disease characterized by intimal thickening from the accumulation of lipids [1], smooth muscle cells, lipid-filled macrophages, monocytes, lymphocytes, erythrocytes, platelets [2–4] and extracellular matrix proteins (collagen, elastin, proteoglycans) [5,6]. It is considered the major contributor to the development of cardiovascular disease, the leading cause of death in the USA [7] and worldwide [8].

Histological studies using excised human vessels and atherosclerotic animal models have provided valuable information regarding the pathophysiology of atherosclerosis and thrombosis. Human vessels collected at autopsy were used by the American Heart Association Committee on Vascular Lesions to stratify the severity of atherosclerotic plaques based on compositional and morphological criteria [9–11]. This classification system was later modified by Virmani et al. [12]. It has also been shown that acute cardiovascular events and sudden death related to atherosclerosis are due to disruption of vulnerable or high-risk plaques and subsequent thrombosis, which may quickly cause luminal occlusion. Conversely, stable plaques can remain clinically asymptomatic. Currently, three distinct histological features: plaque rupture, plaque erosion and calcified nodules, have been associated with luminal thrombosis. Ruptured human plaques, also termed thin-cap atheromas, usually have:

- A thin (<65 µm in the coronary arteries) [13–15], inflamed [16,17] fibrous cap infiltrated by macrophages;
- A large lipid core (>40% of the total lesion area);
- Increased neovessels [18];
- Medial and adventitial disorganization [19];
- Intraplaque hemorrhage [20];
- Positive/outward vessel wall remodeling [21].

Unlike plaque rupture, in eroded plaques the thrombus forms over an intima lacking endothelial cells and a fibrous cap rich in smooth muscle cells, proteoglycans and type 3 collagen fibers [22]. Finally, atherothrombi may also occur as a result of calcified nodules that bulge into the lumen through a disrupted fibrous cap [12].

Despite the incremental understanding of the pathophysiology of atherosclerosis, histological studies are limited by their retrospective nature. Several studies have shown the feasibility of both invasive (angiography, angioscopy, intravascular ultrasound [IVUS], optimal coherence tomography, thermography, Raman spectroscopy, near-infrared spectroscopy) and noninvasive (B-mode ultrasound tomography, CT, PET, MRI) imaging modalities for in vivo vessel wall imaging and characterization of atherosclerosis. Of these techniques, angiography and IVUS have been widely used in clinical practice primarily to estimate the degree of luminal stenosis and stratify patients in different intervention groups. However, angiographic studies of coronary arteries, performed before and after nonfatal myocardial infarction, showed that at
### Table 1. Animal models used in the study of atherosclerosis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Characteristics and use</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammalian nonprimate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice C57BL/6, C3H, BALB/c</td>
<td>The C57BL/6 is the most susceptible strain, the BALB/c has intermediate susceptibility and C3H has the least susceptibility. C57BL/6 mice develop small lesions in the aortic root characterized by lipid-laden macrophages when fed a hyperlipidemic diet for prolonged periods. With further feeding they also develop lesions with cellular debris and collagen.</td>
<td>[163,164]</td>
</tr>
<tr>
<td>ApoB transgenic mice</td>
<td>Develop foam cell-rich lesions when fed a diet enriched in saturated fat and cholesterol.</td>
<td>[165]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Spontaneous lesions form even when the animals are fed a standard chow diet low in fat and free of cholesterol. Lesions rich in foamy macrophages form in the proximal aorta by 3 months of age and more complex lesions develop by 8–9 months. Lesion formation can be significantly accelerated with high-cholesterol HFD. Advanced lesions with fibrous cap, small necrotic cores and lipid deposits form by 3 months of HFD. Lesions are found in the aortic root, the aortic arch, the brachiocephalic artery, the base of the left carotid and the left subclavian arteries and the renal area. Carotid atherosclerosis was induced by using HFD and perivascular constrictive collars. This model was used to study the effects of shear stress on plaque progression and morphology.</td>
<td>[166–168]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/LDLR&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop lesions when the animals are fed a high-fat, high-cholesterol diet. Lesions contained smooth muscle cells, macrophages and T lymphocytes.</td>
<td>[173]</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop foamy lesions when fed an atherogenic diet containing cholesterol, saturated fat and cholate.</td>
<td>[174]</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;−/−&lt;/sup&gt;/ApoBEC&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>A model of human familial hypercholesterolemia.</td>
<td>[175]</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;−/−&lt;/sup&gt;/ApoB&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Exhibit accelerated atherosclerosis on a chow diet. Develop large, complex, lipid-laden atherosclerotic lesions.</td>
<td>[176]</td>
</tr>
<tr>
<td>LDLR&lt;sup&gt;−/−&lt;/sup&gt;/ApoCIII</td>
<td>A model of familial combined hyperlipidemia. Lesions form when the animals are fed an atherogenic diet.</td>
<td>[177]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/LDLR&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop foamy lesions when fed an atherogenic diet containing cholesterol, saturated fat and cholate.</td>
<td>[178]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/C1039G&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Hypercholesterolemia with a mutation in the fibrillin-1 gene leading to impaired elastogenesis promotes features of plaque instability.</td>
<td>[179]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/MMP1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop lesions when fed a high-cholesterol HFD. Surprisingly, the lesions are less advanced after 16 weeks of high-cholesterol HFD.</td>
<td>[180]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/eNOS&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop accelerated atherosclerosis, aortic aneurysm formation and ischemic heart disease.</td>
<td>[181]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/NOS&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop reduced atherosclerosis and have lower plasma lipid peroxides.</td>
<td>[182]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/Ncp1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Develop lesions increased in size and extensive medial degradation. The lesions showed signs of spontaneous plaque disruption with overlay thrombus.</td>
<td>[183]</td>
</tr>
<tr>
<td>Rats</td>
<td>Not a preferred model for studying atherosclerosis. Very resistant to the development of atherosclerosis even when fed with high-cholesterol diets that induce lesions in other species. Induction of atherosclerosis was achieved with a combination of extremely high lipid content coupled to auxiliary procedures such as bile acid supplementation, vascular injury, thyroid destruction and perinephritis.</td>
<td>[184,185]</td>
</tr>
<tr>
<td><strong>Rats</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible, especially the NZW rabbits, to diet-induced atherosclerosis and the type of lesions depend on the duration and composition of the atherogenic diet. Atherosclerotic plaques range from early to advanced/complicated lesions depending on the induction method. Rabbits developed foam cell-rich (fatty steaks) plaques when short-term HFDs (6–10 weeks) were the only stimulus used to induce atherosclerosis. However, intermittent cycles of high-cholesterol feeding with periods of normal diet (2 months of high-cholesterol diet, followed by 2–3 months of normal diet, followed by another cycle of high-cholesterol diet for 2 months and normal diet for 2 months) induced plaques at more advanced stages that resembled human atheroma. Moreover, with the combination of arterial wall injury and hyperlipidemia, advanced lesions form in shorter periods. WHHL rabbits serve as models of homozygous familial hypercholesterolemia. They develop a variety of lesions under normal chow and have been used to study lipoprotein metabolism owing to the elevation of LDLs.</td>
<td>[1,186]</td>
<td></td>
</tr>
</tbody>
</table>

HFD: High-fat diet; IDL: Intermediate-density lipoprotein; LDL: Low-density lipoprotein; MMP: Matrix metalloproteinase; NZW: New Zealand white; VLDL: Very low-density lipoprotein; WHHL: Watanabe heritable hyperlipidemic.
the site of thrombosis, the pre-existing lesion frequently resulted in less than 50% stenosis [23,24] and frequently did not cause angina or a positive treadmill test. Only 20% of acute complete occlusions occur in lesions with a stenosis greater than 75% [25].

Therefore, there is a need for the development of a noninvasive imaging modality that would allow not only the estimation of luminal stenosis but also a compositional characterization of atherosclerotic plaque. This review article will focus on the different animal models currently available for studying atherosclerosis and the applications of noncontrast-enhanced, contrast-enhanced and molecular MRI for preclinical and clinical use.

Animal models of atherosclerosis: advantages & disadvantages

The complexity and slow progression of atherosclerosis in humans and the unpredictable nature of plaque disruption have necessitated the development of animal models for understanding the molecular and cellular pathways involved in disease progression and the clinical manifestations, as well as the development of diagnostic procedures and therapeutic interventions. Unlike in humans, animal models allow the development of the disease in a reasonable time span and under precise settings where environmental, genetic and dietary variables can be controlled. Furthermore, animals allow the evaluation of risk factors independently or in combinations, in the presence or absence of other intercurrent diseases. Many requirements need to be satisfied in order to make an animal model suitable for the study of atherosclerosis. Some of the factors include: strain availability, susceptibility to disease, ease in handling, breeding and maintenance cost, reproducibility of results, anatomical, morphological and biochemical similarities to the human disease.

Anitschkow and Chalatow were among the first researchers to induce experimental atherosclerosis in animals by feeding rabbits an enriched cholesterol diet [1,26]. Since then, several other experimental conditions have been used to induce lesions in different animal species including dietary, physical, chemical, immunological and transgenic approaches applied individually or in combinations, simultaneously or sequentially. A summary of the different animal models available for studying atherosclerosis together with their basic characteristics and uses is illustrated in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Characteristics and use</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian nonprimate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbits (cont.)</td>
<td>St Thomas’ strain of familial combined hyperlipidemia develops atherosclerotic lesions on a standard chow diet and are characterized by elevated lower-density lipoproteins (VLDL, IDL, LDL)</td>
<td>[196]</td>
</tr>
<tr>
<td></td>
<td>Jackson Laboratory AX/JU strains are hyper-responsive to dietary cholesterol</td>
<td>[197]</td>
</tr>
<tr>
<td></td>
<td>Jackson Laboratory IIIVO/JU strain is hypo-responsive to dietary cholesterol</td>
<td>[198,199]</td>
</tr>
<tr>
<td></td>
<td>Transgenes of different human apolipoproteins have been expressed in NZW and WHHL rabbits for the study of lipoprotein metabolism</td>
<td>[199]</td>
</tr>
<tr>
<td></td>
<td>Transgenic rabbit model of MMP-12 in atherosclerosis was used to study the effects of MMP in plaque formation and progression</td>
<td>[200]</td>
</tr>
<tr>
<td>Swine</td>
<td>Susceptible to dietary induced atherosclerosis. Lesions occur in both the aorta and branch vessels. The size of heart and vessels is sufficient for studies of cardiovascular function, ischemic heart disease and for developing new diagnostic and surgical procedures</td>
<td>[201–204]</td>
</tr>
<tr>
<td></td>
<td>Yucatan miniature swine breed is also susceptible to high-fat, high-cholesterol, diet-induced atherosclerosis with and without the presence of diabetes</td>
<td>[204–206]</td>
</tr>
<tr>
<td></td>
<td>Diabetes in conjunction with hyperlipidemia was used in Yorkshire swine to accelerate atherosclerosis</td>
<td>[207]</td>
</tr>
<tr>
<td></td>
<td>Genetic mutations affecting the structure of plasma lipoproteins or the LDL receptor have been used to induce hypercholesterolemia and atherosclerosis in the coronary arteries</td>
<td>[208–210]</td>
</tr>
<tr>
<td></td>
<td>A familial hypercholesterolemic downsized pig with human-like coronary atherosclerosis has been proposed as a model for preclinical studies</td>
<td>[211]</td>
</tr>
<tr>
<td>Dogs</td>
<td>Cholesterol feeding and thyroid inactivation for a year (using thiouracil) are needed to induce advanced lesions</td>
<td>[212]</td>
</tr>
<tr>
<td></td>
<td>Addition of butter in cholesterol–thiouracil diet accelerates disease progression and foamy lesions form by 8 weeks</td>
<td>[213]</td>
</tr>
</tbody>
</table>

HFD: High-fat diet; IDL: Intermediate-density lipoprotein; LDL: Low-density lipoprotein; MMP: Matrix metalloproteinase; NZW: New Zealand white; VLDL: Very low-density lipoprotein; WHHL: Watanabe heritable hyperlipidemic.
Table 2. *In vivo* MRI of atherosclerosis in animal models.

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Vessel</th>
<th>Target</th>
<th>Contrast agent</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mice</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Abdominal aorta and iliac arteries</td>
<td>None</td>
<td>Non-CE</td>
<td>[36]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aorta</td>
<td>None</td>
<td>Non-CE</td>
<td>[37]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Thoracic aorta</td>
<td>None</td>
<td>Non-CE</td>
<td>[38]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic root</td>
<td>None</td>
<td>Non-CE</td>
<td>[39]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Plaque regression in the thoracic aorta</td>
<td>None</td>
<td>Non-CE</td>
<td>[40]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Injury-induced neointima formation in the carotid artery</td>
<td>None</td>
<td>Non-CE</td>
<td>[41]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Abdominal aorta</td>
<td>None</td>
<td>P717, gadolinium-based blood pool agent</td>
<td>[214]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic arch</td>
<td>None</td>
<td>P792 (Vistarem&lt;sup&gt;™&lt;/sup&gt;), gadolinium-based blood pool agent</td>
<td>[215]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic arch and aortic root</td>
<td>VCAM-1</td>
<td>Multimodal nanoparticles</td>
<td>[84,85]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Abdominal aorta</td>
<td>Macrophage scavenger receptor</td>
<td>Gadolinium-loaded immunomicelles and bimodal PEG-micelles</td>
<td>[106,107]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic root <em>ex vivo</em></td>
<td>Oxidation-specific epitopes</td>
<td>Gadolinium-loaded micelles</td>
<td>[114]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic arch</td>
<td>MMP</td>
<td>P947 gadolinium based</td>
<td>[112]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Abdominal aorta</td>
<td>Lipoproteins</td>
<td>LDL-based nanoparticles (GdDO3A-OA-LDL)</td>
<td>[110,111]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Brachiocephalic</td>
<td>Elastin</td>
<td>Small molecular weight gadolinium-based peptide</td>
<td>[148]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic arch and abdominal aorta</td>
<td>Annexin-5</td>
<td>Gadolinium-loaded micelles</td>
<td>[152]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Brachiocephalic</td>
<td>None</td>
<td>SPIO</td>
<td>[98]</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;/eNOS&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Abdominal aorta</td>
<td>Cannabinoid receptor and NGAL</td>
<td>Gadolinium-loaded micelles</td>
<td>[108,109]</td>
</tr>
<tr>
<td>LDL&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Brachiocephalic</td>
<td>None</td>
<td>Non-CE</td>
<td>[216]</td>
</tr>
<tr>
<td>LDL&lt;sup&gt;−/−&lt;/sup&gt;/LOX-1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Aortic root and arch</td>
<td>LOX-1</td>
<td>Gadolinium labeled LOX-1 antibody</td>
<td>[113]</td>
</tr>
<tr>
<td>C57/B6J</td>
<td>Carotid thrombi</td>
<td>α2-antiplasmin</td>
<td>Bimodal α2-antiplasmin</td>
<td>[147]</td>
</tr>
<tr>
<td><strong>Rabbits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta and thrombosis</td>
<td>None</td>
<td>Non-CE</td>
<td>[42–50]</td>
</tr>
<tr>
<td>WHHL</td>
<td>Abdominal aorta</td>
<td>None</td>
<td>Non-CE</td>
<td>[51]</td>
</tr>
<tr>
<td>NZW</td>
<td>Coronary arteries</td>
<td>None</td>
<td>Non-CE</td>
<td>[52]</td>
</tr>
<tr>
<td>NZW and WHHL</td>
<td>Abdominal aorta</td>
<td>None</td>
<td>Gadofluorine-M (blood pool agent)</td>
<td>[217–221]</td>
</tr>
<tr>
<td>WHHL</td>
<td>Abdominal aorta</td>
<td>None</td>
<td>Gadopentetate dimeglumine</td>
<td>[121]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta</td>
<td>None</td>
<td>Gadopentetate dimeglumine</td>
<td>[120,122]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta thrombi associated with plaque disruption</td>
<td>Fibrin</td>
<td>EP-2104R</td>
<td>[134]</td>
</tr>
<tr>
<td>NZW</td>
<td>Carotid artery thrombi (external injury and stasis)</td>
<td>Fibrin</td>
<td>EP-2104R</td>
<td>[222]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta</td>
<td>MMP</td>
<td>P947 is gadolinium-based</td>
<td>[223]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta</td>
<td>Blood albumin</td>
<td>Gadofosveset</td>
<td>[126]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta</td>
<td>Blood albumin</td>
<td>B-22956/1</td>
<td>[224]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta</td>
<td>MPO</td>
<td>Bis-SHT-DTPA(Gd)</td>
<td>[151]</td>
</tr>
<tr>
<td>NZW</td>
<td>Abdominal aorta</td>
<td>Angiogenesis</td>
<td>α,β&lt;sub&gt;3&lt;/sub&gt;-integrin-targeted nanoparticles</td>
<td>[123,124]</td>
</tr>
<tr>
<td>NZW</td>
<td>Thoracic aorta</td>
<td>None</td>
<td>USPIO</td>
<td>[88]</td>
</tr>
</tbody>
</table>

CE: Contrast enhanced; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; LDLR: Low-density lipoprotein receptor; MION: Monocrystalline iron oxide nanoparticle; MMP: Matrix metalloproteinase; MPIO: Microparticles of iron oxide; MPO: Myeloperoxidase; NZW: New Zealand white; PEG: Polyethylene glycol; SPIO: Superparamagnetic iron oxide particles; SPION: Superparamagnetic iron oxide nanoparticle; USPIO: Ultrasmall superparamagnetic iron oxide particles; WHHL: Watanabe heritable hyperlipidemic.


MRI of atherosclerosis in animal models & humans

Over the last decades extensive research has been dedicated to developing MR methods for in vivo imaging of atherosclerosis in animal models and humans. The major applications of MRI in characterizing animal and human atherosclerosis are described below and are summarized in Tables 2 & 3.

Assessment of plaque burden & composition

MRI has been applied to characterize plaque composition on the basis of biophysical and biochemical factors (T₁ and T₂ relaxation times), proton density, physical state, molecular motion, fibrous protein content (magnetization transfer) and diffusion coefficients (diffusion-weighted imaging) both in vivo and ex vivo [27–36]. In addition, in vivo techniques such as the black-blood pulse sequence and the use of phased-array receiver coils have improved the delineation of the arterial lumen from the vascular wall, which is critical for lesion visualization [37]. Validation studies were first performed in experimental models including mice [36–41], cholesterol-fed rabbits [42–52] and pigs [53]. In humans, validation of the in vivo MRI findings was performed mainly by using ex vivo carotid endarterectomy specimens. Several studies showed that the combination of multiple MR contrast weightings (proton density, T₁-weighted, T₂-weighted and time of flight) can be used to identify plaque components [54–59] based on their relative signal intensities and relaxation times. Multicontrast in vivo MRI has been used to evaluate plaque size [60] and components including the lipid core, fibrous cap, calcification [54,55,61–64], intraplaque hemorrhage [65,66] as well as features associated with symptomatic human carotid plaques [67]. Furthermore, diffusion-weighted imaging is another technique used to generate contrast between plaque components based on the characteristic diffusion coefficients of water in each tissue [68,69]. Lastly, magnetization transfer between restricted and free-water protons was also used to discriminate the collagenous fibrous cap and the media from the lipid core and adventitia [70].

Several contrast agents have been used to improve the conspicuity of atherosclerotic plaques. Contrast-enhanced MRI using gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) has been used to increase the sensitivity of MRI and further improve the identification of plaque components. Gd-DTPA has been used for the discrimination between the fibrous cap and the lipid core [71–73] and the visualization of coronary atherosclerosis [74–76].

MRI and MR angiography of coronary arteries still remains challenging owing to cardiac motion, the small caliber and the tortuous structure of the vessels. However, advanced pulse sequence...
design with navigator gating, with and without breath-holds, has allowed the visualization of the coronary lumen and vessel wall [77–86]. For example, coronary MRI of asymptomatic Type 1 diabetics revealed greater coronary plaque burden in subjects with nephropathy compared with those with normoalbuminuria (Figure 1) [82].

### Assessment of endothelial activation & permeability

Increase in endothelial permeability and upregulation of adhesion molecules (VCAM-1, ICAM-1, P-selectin) on the endothelial surface occurs in the early stages of atherosclerosis. Increased endothelial leakage allows blood molecules such as low-density lipoprotein (LDL) particles to passively diffuse into the vessel wall whereas expression of adhesion molecules is responsible for the receptor-mediated recruitment of leukocytes. Recently, gadofosveset, a gadolinium-based agent that reversibly binds to blood albumin has been shown to be associated with damaged endothelial cells in a swine model of coronary injury (Figure 2) [83]. Furthermore, multimodal nanoparticles (VIPN-28) [84,85] and microparticles of iron oxide [86] targeting the VCAM-1 receptor and/or P-selectin have been used to image activated endothelium in mouse atherosclerotic plaques. Interestingly, a recent study showed that plaque permeation by contrast agents was strongly dependent on particle size [87].

### Assessment of plaque macrophages & lipoproteins

Macrophages are key players in the initiation, progression and complication of atherosclerosis. Superparamagnetic iron oxide particles of different sizes stabilized with

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**Table 3. MRI of atherosclerosis in humans.**

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Target</th>
<th>Contrast agent</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid</td>
<td>None</td>
<td>Non-CE</td>
<td>[60–65,67,73,226–239]</td>
</tr>
<tr>
<td>Carotid</td>
<td>None</td>
<td>Gadopentetate dimeglumine</td>
<td>[71,115,116,118,240–244]</td>
</tr>
<tr>
<td>Carotid</td>
<td>None</td>
<td>Gadofosveset</td>
<td>[125]</td>
</tr>
<tr>
<td>Carotid</td>
<td>None</td>
<td>USPIO</td>
<td>[99–105]</td>
</tr>
<tr>
<td>Carotid</td>
<td>None</td>
<td>Non-CE, direct thrombus imaging</td>
<td>[313,245]</td>
</tr>
<tr>
<td>Carotid/aorta</td>
<td>None</td>
<td>EP-2104R, thrombus imaging</td>
<td>[344]</td>
</tr>
<tr>
<td>Aorta</td>
<td>None</td>
<td>Non-CE</td>
<td>[246–249]</td>
</tr>
<tr>
<td>Aorta</td>
<td>None</td>
<td>Gadopentetate dimeglumine</td>
<td>[72]</td>
</tr>
</tbody>
</table>

**Coronary**

| MRI         | None   | Non-CE                          | [35,156,250–254]     |
| MRI         | None   | Gadopentetate dimeglumine       | [74–76]              |
| MRI         | None   | Non-CE, direct thrombus imaging | [255]                |
| MRA         | None   | Non-CE                          | [77–81]              |
| MRA         | None   | MS-325/AngioMARK (intravascular agent) | [256]             |

CE: Contrast enhanced; MRA: Magnetic resonance angiography; USPIO: Ultrasmall superparamagnetic iron oxide particles.

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**Figure 1. MRI and MR angiography of coronary arteries in patients with Type 1 diabetes.** 3D reformatted coronary MRI of the proximal RCA in two subjects without coronary luminal stenosis: a 58-year-old man with long-standing Type 1 diabetes and normoalbuminuria (A) and a 44-year-old man with long-standing Type 1 diabetes and diabetic nephropathy. (C) The corresponding 3D black-blood vessel wall scans show no cardiovascular MRI evidence of atherosclerotic plaque; (B) average and maximum vessel wall thickness (1.1 and 1.3 mm, respectively) and an increased atherosclerotic plaque burden; (D) average and maximum vessel wall thickness (2.3 and 3.0 mm, respectively). The anterior and posterior RCA walls are indicated by arrows [82]. RCA: Right coronary artery.
different surface-coating materials (e.g., dextran or citrate) have been used to estimate the macrophage content of atherosclerotic plaques by becoming nonspecifically endocytosed by macrophages in hyperlipidemic rabbits [88–97], mice [98] and human carotid plaques [99–105]. Macrophages have also been imaged by using gadolinium-loaded micelles targeting the macrophage scavenger receptor in mouse plaques [106,107]. Atherosclerotic plaque macrophages also express the peripheral cannabinoid receptor (CB2-R) and promote fibrous cap degradation by secretion of NGAL. CB2-R- and NGAL-targeted gadolinium-loaded micelles were shown to enhance murine atherosclerotic plaques with a vulnerable phenotype [108,109]. Gadolinium-loaded recombinant high-density lipoprotein-like nanoparticles [110,111] and LDL-based nanoparticles (GdDO3A-OA-LDL) [112] have also been developed to image atherosclerosis in mice. Furthermore, LOX-1 and oxidized plaque LDL particles have been imaged using antibodies that bind to LOX-1 receptor [113] andoxidation specific epitopes [114], respectively.

Assessment of plaque neovascularization

Aoki et al. were the first to observe a band of enhancement corresponding to the outer vessel wall, after injection of Gd-DTPA, which was attributed to angiogenesis of the wall itself [115]. Enhancement of the outer rim was minimal in early phases of the disease and gradually increased. Subsequently, several other studies have demonstrated a correlation between Gd-DTPA uptake and plaque neovascularization, inflammation, endothelial permeability and fibrosis both in human [74,76,116–119] and animal models [117,120–122]. Gadolinium-based nanoparticles that target $\alpha_\beta_3$ integrins have also been
engineered to selectively image plaque angiogenesis and as vehicles for antiangiogenic drug delivery in rabbit aortas [123,124]. Recently, the uptake of gadofosveset was shown to correlate with neovessel density in human carotid [125] and rabbit aortic plaques [126].

Assessment of plaque intraplaque hemorrhage & thrombus
Intraplaque hemorrhage and thrombosis are major components of plaque vulnerability. Most MRI studies have focused on the detection of hematoma [127,128], venous thrombosis [129,130], intraplaque hemorrhage [131] and arterial thrombi [132,133] based on the temporal changes of T1 and T2 relaxation of different oxygenation states of hemoglobin in erythrocytes. Subsequently, the conspicuity of thrombi has been significantly increased by using fibrin- (Figure 3; rabbit model) [134–144], platelet- [97,145,146] and α2-antiplasmin-targeting contrast agents [147].

Assessment of plaque extracellular matrix
The fine-tuned balance in the production and degradation of extracellular matrix proteins (collagen, elastin, proteoglycans) is essential for plaque development and stability. Recently, with the development of a small molecular weight, gadolinium-based, elastin-targeting contrast agent, MRI of the vessel wall at all stages of atherosclerosis has become feasible in mouse atherosclerotic plaques (Figure 4) [148] and in a swine model of coronary injury [149].

Assessment of plaque enzymatic activity & apoptosis
Activated matrix metalloproteinases degrade the extracellular matrix and weaken the fibrous cap leading to plaque vulnerability. In vivo and ex vivo MRI for the characterization for matrix metalloproteinase-rich plaques was achieved with the use of a gadolinium-based matrix metalloproteinase-sensitive MRI contrast (P947) [150].

Figure 4. In vivo assessment of plaque burden by morphometric measurements. (A) Cross-sectional views of brachiocephalic arteries by MRI of control and ApoE−/− mice 4, 8 and 12 weeks after the onset of HFD (n = 8 per group). High-resolution DE images overlaid on time-of-flight images with corresponding sections from histology (H&E and EvG stain). (B) Comparison of average PAMV, calculated from morphometric measurement on high-resolution DE images after the injection of elastin-specific MR contrast agent (n = 8 per group). (C & D) Scatter plots showing significant (p < 0.05) correlation between morphometric PAMV measurements (C) and lumen cross-sectional area measurements (D) on high-resolution DE-MRI images and on corresponding EvG-stained histological sections (n = 15). Scale bars: white, 250 µm; black, 100 µm. Values are expressed as means ± standard deviation [148].

Myeloperoxidase is another important enzyme secreted by activated macrophages at multiple stages of plaque development. Recently, in vivo MRI of myeloperoxidase has been achieved with the development of the gadolinium-based myeloperoxidase sensor bis-SHT-DTPA(Gd) in rabbit atherosclerotic plaques [55]. Lastly, cellular apoptosis is also a key feature of plaque progression and stability. Imaging of apoptosis has been shown in atherosclerotic mice using gadolinium-loaded micelles targeting annexin-5 [52].

**Assessment of vascular remodeling**

Positive remodeling has been recognized as a possible mechanism to alleviate luminal narrowing based on autopsy studies [153–155]. In previous in vivo MRI studies of patients with subclinical coronary atherosclerosis [156,157] and of Watanabe hypercholesterolemic rabbits [121], positive remodeling was observed as an increase in the vessel wall area, determined by the outer vessel wall contour, with concurrent preservation of the lumen area. More recently, MRI characterization of vessel wall remodeling and its association with plaque vulnerability, using standardized cut off values, has been shown in atherosclerotic rabbits (Figure 5) [122].

**Conclusion & future perspective**

Noncontrast-enhanced, contrast-enhanced and molecular MRI of various biological processes in atherosclerosis have been successfully demonstrated in small and large animal models as well as human subjects. The use of animal models allows the development of new imaging approaches to better understand atherosclerosis and its progression. Future studies will be focused on improving the sensitivity and specificity of MRI for the detection of vulnerable plaques and the assessment of the impact of therapeutic interventions on plaque biology.
protocols, contrast agents and therapeutic interventions in a controlled fashion. Furthermore, it provides specimens for *ex vivo* validation studies. The noninvasive nature of MRI, the high spatial resolution and the lack of ionizing radiation make MRI an advantageous imaging modality for both preclinical and clinical studies. The development of higher field scanners and dedicated coils that allow for higher signal:noise ratio, the incorporation of multiple elements in the coils that allow higher acceleration factors, and the ongoing development of pulse sequences can significantly improve the diagnostic performance of MRI and allow translation of the knowledge derived from preclinical studies to imaging of the human disease. The ultimate goal of *in vivo* MRI of atherosclerosis is to reliably and prospectively identify plaques at higher risk for disruption that could improve medical decision making and patient outcome.

Currently, the use of most new contrast agents has been limited to preclinical models for investigating imaging protocols and elucidating the underlying biological processes involved in disease progression in a longitudinal noninvasive manner. Despite the exciting and promising results derived from the preclinical studies very few of these agents progressed to the clinical setting [158,159]. Important limitations that impede the translation to the clinical arena include scalability, cost, safety, favorable pharmacokinetics and regulatory guidelines [160]. Recently, two major prospective clinical studies that examined coronary atherosclerotic vessels in humans revealed that independent predictors including a large plaque burden, a small lumen area and a thin cap fibroatheroma (PROSPECT study) [161] and remodeling index (VIVA study) [162] were associated with future major adverse cardiac events as classified by radiofrequency IVUS. As shown in this review, similar measurements have been derived with native noncontrast and molecular MRI both in a preclinical and clinical setting. Although IVUS has superior spatial resolution compared with MRI it is invasive and therefore not suitable as a screening method. To this end, we envision the future use of noncontrast and molecular MRI as a noninvasive test for risk assessment and monitoring of interventions in subjects with suspected atherosclerosis by morphologic and biological plaque characterization.

### Executive summary

**Background**

- Atherosclerosis and its thrombotic complications are considered the major contributor to the development of acute cardiovascular symptoms.
- Histological studies have added indispensable knowledge to our understanding of the pathophysiology of atherosclerosis but they are limited by their retrospective nature.
- Several invasive and noninvasive imaging modalities have shown the feasibility of *in vivo* vessel wall imaging for the characterization of atherosclerosis.

**Animal models of atherosclerosis**

- The complexity and slow progression of atherosclerosis in humans and the unpredictable nature of thrombotic events have necessitated the development of several animal models.
- Although no perfect animal model exists, each animal model can be used to address specific biological questions.
- The use of animal models has broadened our understanding of the molecular and cellular pathways involved in disease progression and its clinical complications, the development of new imaging modalities, contrast agents and therapeutic interventions in a controlled fashion.

**MRI of atherosclerosis in animal models & humans**

- MRI has evolved as one of the leading noninvasive imaging modalities to visualize the vessel wall with high spatial resolution and without ionizing radiation, making it suitable for both preclinical and clinical studies.
- Noncontrast-enhanced, contrast-enhanced and molecular MRI of various biological processes in atherosclerosis has been successfully demonstrated in small and large animal models as well as human subjects.
- Currently, MRI can be used to assess plaque burden and composition, endothelial activation and permeability, plaque enzymatic activity and apoptosis, macrophages and lipoproteins, neovascularization, intraplaque hemorrhage and thrombus, extracellular matrix and vascular remodeling.

**Conclusion & future perspective**

- The noninvasive nature of MRI, the high spatial resolution and the lack of ionizing radiation make MRI an advantageous modality for imaging atherosclerosis.
- The ongoing optimization of both MRI hardware and software can significantly improve the diagnostic performance of MRI and allow us to translate the knowledge derived from preclinical studies to imaging of the human disease.
- The ultimate goal of *in vivo* MRI of atherosclerosis is to reliably and prospectively identify plaques at higher risk of disruption that could improve medical decision making and patient outcome.
Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.
No writing assistance was utilized in the production of this manuscript.

References
Papers of special note have been highlighted as:
* of interest
** of considerable interest
** One of the first publications demonstrating that cholesterol-rich diets induce experimental atherosclerosis in rabbits.
** Provided histological evidence that plaque rupture was the underlying cause of most acute cardiovascular events in subjects that have died suddenly.
17 van der Wal AC, Becker AE, van der Loos C, Das P. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 89, 36–44 (1994).
** Provided histological evidence that plaque erosion without rupture can cause thrombosis leading to sudden death in humans.
**REVIEW**

Phinikaridou & Botnar


**Demonstrated the usefulness of the black-blood pulse sequence for delineation of the vessel wall from the blood and the visualization of atherosclerosis.**


**Initial demonstration of the feasibility to image experimental atherosclerosis in rabbits *in vivo*.**


**In vivo identification of thrombosis associated with plaque disruption in rabbits.**


MRI of atherosclerosis: from mouse to man

**The application of the multicontrast MRI approach to identify different plaque components.**


**Application of advanced pulse sequences for coronary MR angiography.**


**Application of advanced pulse sequences for coronary MR angiography.**


**Application of advanced pulse sequences.**


REVIEW

Philkarioudou & Botnar


• Showed that dynamic contrast-enhanced MRI provides an indication of the extent of neovascularure within carotid atherosclerotic plaque.


Shaw et al. Human coronary arteries enlarge in relation to plaque area and that luminal stenosis may be delayed until the lesion occupies 40% of the internal elastic lamina area.


Shaw et al. Coronary MRI can be used to detect a Glagow type of arterial remodeling in vivo.


Constantinides P, Booth J, Carlson G. Production of advanced cholesterol atherosclerosis in the rabbit. *Arch. Pathol. 70, 80–92 (1960).*


235 Saam T, Underhill HR, Chu B et al. Prevalence of American Heart Association type 6 carotid atherosclerotic lesions identified by magnetic resonance imaging for different levels of stenosis as measured by duplex ultrasound. J. Am. Coll. Cardiol. 51(10), 1014–1021 (2008).

* Prospective investigation of asymptomatic individuals with 50–79% stenosis; provided evidence that the size of the lipid-rich necrotic core may govern the risk of future surface disruption.


** Application of advanced pulse sequences for coronary vessel wall MRI.


** Application of advanced pulse sequences for coronary vessel wall MRI.