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HDL as a contrast agent for medical imaging

David P Cormode¹, Juan C Frias¹, Yanqing Ma², Wei Chen¹, Torjus Skajaa^{1,3}, Karen Briley-Saebo¹, Alessandra Barazza^{1,2}, Kevin Jon Williams⁴, Willem JM Mulder¹, Zahi A Fayad^{1,*}, and Edward A Fisher^{2,†,‡}

¹Mount Sinai School of Medicine, NY, USA

²Department of Medicine (Cardiology), Marc & Ruti Bell Vascular Biology & Disease Program and the NYU Center for the Prevention of Cardiovascular Disease, New York University School of Medicine, New York University, Smilow 8 522 First Ave. New York, NY 10016, USA

³Århus University, Denmark

⁴Thomas Jefferson University, PA, USA

Abstract

Contrast-enhanced MRI of atherosclerosis can provide valuable additional information on a patient's disease state. As a result of the interactions of HDL with atherosclerotic plaque and the flexibility of its reconstitution, it is a versatile candidate for the delivery of contrast-generating materials to this pathogenic lesion. We herein discuss the reports of HDL modified with gadolinium to act as an MRI contrast agent for atherosclerosis. Furthermore, HDL has been modified with fluorophores and nanocrystals, allowing it to act as a contrast agent for fluorescent imaging techniques and for computed tomography. Such modified HDL has been found to be macrophage specific, and, therefore, can provide macrophage density information via noninvasive MRI. As such, modified HDL is currently a valuable contrast agent for probing preclinical atherosclerosis. Future developments may allow the application of this particle to further diseases and pathological or physiological processes in both preclinical models as well as in patients.

Keywords

contrast agent; HDL; lipoproteins; medical imaging; MRI; nanoparticle

The noninvasive imaging of atherosclerotic plaques by MRI has obvious preclinical and clinical applications. In addition to diagnostic applications, noninvasive imaging would also allow the assessment of the responses to therapies [1]. In the preclinical setting, an immediate obstacle for MRI of atherosclerosis is the small size of the arteries of the most common model of the human disease, namely genetically engineered mice. The mice are made deficient in a lipoprotein clearance factor, such as ApoE or the LDL receptor, which results in elevated plasma levels of the atherogenic (non-HDL) lipoproteins. If the mice are placed on a cholesterol- and fat-enriched diet (the so-called 'Western diet'), plasma non-HDL levels are further elevated and atherosclerotic plaque progression accelerates [2]. In the mid-1990s, we were successful in demonstrating that noncontrast-enhanced MRI could detect mouse aortic atherosclerosis [3], which led to a series of papers demonstrating both practical applications [4] and limitations [5].

The extension of this initial success was strongly influenced by our previous experience in reconstituting HDL [6]. It was decided to convert human HDL to an MRI contrast-enhancing agent. This would not only be technically feasible, but would have the advantage of adapting a nanoparticle that is small enough (~10 nm) to naturally cross the endothelium to enter plaques. Furthermore, HDL has a number of important interactions with plaque components [7], indicating the likelihood of this nanoparticle gathering to some extent in atherosclerotic tissue. Experiments reported by Shaish *et al.* using radiolabeled HDL demonstrated, via autoradiography, that this particle localized in the aortas of ApoE-deficient (ApoE^{-/-}) mice [8], further encouraging our strategy. Gadolinium is widely used to generate contrast for MRI and was available in a form in which it was chelated to a diethylene triamine pentaacetic acid-phosphatidyl ethanolamine (DTPA-PE) molecule, which could be included in the phospholipids layer of HDL. Therefore, an integrated gadolinium-HDL particle was proposed as an initial MRI contrast agent for atherosclerosis.

First-generation HDL nanoparticles

The first gadolinium-labeled HDL was formed by reconstituting human HDL with gadolinium (Gd)-DTPA-PE and also with a fluorescent dye, NBD, which would allow convenient *ex vivo* analyses of the distribution of the particle in the plaque [9]. The labeled HDL we termed Gd-HDL. The reconstitution procedure followed the classical cholate dialysis method, with the components used including phosphatidyl choline and ApoA-I to form the surface and cholesteryl ester to form the core [9]. These spherical Gd-HDL particles were then injected into ApoE^{-/-} mice that had aortic atherosclerosis. As shown in Figure 1, there was obvious enhancement in the signal intensity in the aorta of these mice relative to images acquired preinjection. The tissue analysis showed the fluorescent dye frequently within the plaque macrophages, suggesting that the Gd-HDL was naturally targeted to plaque macrophages.

In an extension of the first set of studies, Frias *et al.* then reconstituted HDL-disks, using the full-length human ApoA-I protein and phospholipids, incorporating Gd-DTPA-PE and NDB, with equivalent imaging results obtained [10]. Recently, a variation of this nanoparticle was reported, where the Gd-DTPA-PE lipid was replaced with the Gd-AAZTA-C17 lipid [11]. This lipid allows two water molecules to bind to gadolinium [12], whereas only one water molecule can bind to gadolinium with the Gd-DTPA-PE lipid. As a consequence, the Gd-AAZTA-C17-labeled HDL has a higher longitudinal relaxivity (a measure of MRI contrast-generating potential) than the original HDL formulations by a factor of four, making it a more efficacious contrast agent.

This basic platform structure was then modified, as described later, to improve targeting to macrophages, to simplify the reconstitution using sonication and ApoA-I peptide mimetics, and to incorporate imaging agents into the core as well as the surface in order to create multimodal imaging agents.

Enhanced macrophage targeting of HDL

An alternative version of gadolinium-labeled HDL was reported by Chen *et al.*, where an ApoE-derived peptide (P2A2) was incorporated into the phospholipid layer of the particle, termed P2A2-HDL [13]. The sequence of this peptide was derived from the LDL-receptor-binding domain of ApoE, and it has been shown to facilitate cellular uptake of nanoparticles [14]. The aim of including this peptide was to improve the accumulation of the particle in atherosclerotic tissue by exploiting the interactions of the ApoE sequence with macrophages. The uptake of P2A2-HDL was compared with Gd-HDL in J774A.1 macrophages *in vitro*, using fluorescence measurements and MRI. Both techniques revealed increased uptake of the P2A2-HDL nanoparticles in these macrophage cells. P2A2-HDL was then applied to ApoE^{-/-} mice and sequential MR scanning was performed. The new formulation produced greater contrast than

the original HDL formulation in the aortas of these mice, indicating that the P2A2 peptide had the desired effect *in vivo*. This was despite the half-life of P2A2-HDL being approximately a third of the half-life of Gd-HDL, likely due to the cationic nature of the P2A2 peptide or due to clearance via the LDL receptor in the liver. Confocal microscopy demonstrated this peptide-modified HDL contrast agent to still be taken up in macrophages in the aorta.

Peptide-based HDL contrast agents

Anantharamaiah and other workers have performed substantial research on synthetic peptides that mimic the properties of ApoA-I [15]. The motivation for this work is to understand the structural properties of ApoA-I that are key to HDL function and to generate candidate peptides that can be used to artificially raise HDL levels. The sections of ApoA-I that embed into the phospholipid coating of HDL are amphipathic α -helices [16]. The hydrophobic portions of these α -helices interact with the acyl chains of the phospholipids while the hydrophilic faces are exposed to the aqueous medium. The basis of the series of peptides that has been synthesized to mimic ApoA-I is an 18 amino acid sequence (DWL-KAF-YDK-VAE-KLK-EAF), known as 18A [17], which forms an amphipathic α -helix (Figure 2A). Peptides with variants of this sequence have been demonstrated to mimic various properties of ApoA-I, such as forming complexes with phospholipids of the same size and morphology as HDL [17], eliciting cholesterol efflux from macrophages [18], activating the plasma enzyme lecithin-cholesterol acyltransferase (LCAT) [19] and binding to macrophage membrane proteins, such as ABCA1 [20].

Cormode *et al.* have reported the synthesis of a HDL-based contrast agent where the peptide 37pA was used in place of ApoA-I (Figure 2B) [21]. 37pA is composed of two 18A sequences joined by a proline residue. The motivation behind this work was to investigate whether artificial peptide-based HDL would also perform as a plaque-specific contrast agent. HDL-based contrast agents using synthetic peptides could be advantageous as the absence of plasma-derived products in the formulation could be preferable for use in patients. Peptide-based HDL-like contrast agents, which we will refer to as 37pA-Gd, were synthesized via hydration of a mixed phospholipids film with a solution of 37pA followed by sonication, filtration and concentration. Some of these phospholipids were gadolinium labeled or fluorophore labeled, as before. The particles thus formed were characterized via dynamic light scattering, transmission electron microscopy (TEM), fast protein liquid chromatography, gel electrophoresis, relaxometry and various compositional techniques. The size measurements showed 37pA-Gd to have an average diameter of approximately 8 nm, within the size range of native HDL. TEM imaging revealed the particles to have a disc-like structure similar to complexes formed between ApoA-I and phospholipids. The longitudinal relaxivity of these nanoparticles was $9.8 \text{ mM}^{-1}\text{s}^{-1}$ at 1.5 T – between two- and three-times higher than that of commercially available Gd-based contrast agents. Compositional investigations showed the inclusion of the feedstock materials to be highly efficient. Interestingly, when 37pA-Gd was tested for macrophage cholesterol efflux it exhibited significant activity in this important measure of HDL function (Figure 2C). A phospholipid film was hydrated without 37pA to create particles we term Gd-micelles, which were used as a control.

37pA-Gd was tested as an atherosclerosis-specific contrast agent in ApoE^{-/-} mice using a 9.4 T small-animal scanner. At 24 h postinjection of 37pA-Gd the signal intensity in the aorta of the ApoE^{-/-} mice increased by an average of 94%, as compared with preinjection images. Typical examples of these images are shown in (Figure 2D). This heightened intensity slowly decreased over time, returning to baseline after 10 days. When 37pA-Gd was tested in nonatherosclerotic, wild-type mice or when Gd-micelles were tested in ApoE^{-/-} mice the increases in intensity in aortas were not significant. This demonstrated the specificity of 37pA-Gd for atherosclerotic tissue. As in the work previously discussed, confocal microscopy was

performed on the aorta of mice injected with the contrast agent. This confirmed the localization of 37pA-Gd in the aorta and showed this agent to be macrophage specific, as was the case for native HDL-based contrast agents (Figure 2E). This study demonstrated, therefore, that 37pA can be used in place of ApoA-I in HDL-based contrast agent formulations for atherosclerosis imaging.

More recently, a similar synthetic HDL formulation was reported where 18A was used in place of ApoA-I (termed 18A-Gd) and compared to 37pA-Gd [22]. Uptake of these particles by macrophages *in vitro* was monitored via fluorescence imaging techniques. Both formulations were taken up at a much greater rate than Gd-micelles and the uptake was decreased under competition-inhibition conditions. Furthermore, the uptake increased only slowly at concentrations over 0.04 mM Gd. Taken together, these results indicate that both 18A-Gd and 37pA-Gd are taken up by macrophages in a saturable, receptor-like manner. The differences in uptake rate were not found to be significant in this setting. *In vivo* experiments with ApoE^{-/-} mice showed that the contrast produced in the aorta, macrophage localization, pharmacokinetics and biodistribution of the two agents was also similar, and it was concluded that 18A-based HDL contrast agents are also efficacious for macrophage detection by MRI.

Nanocrystal core HDL

While inclusion of contrast-generating materials in the phospholipid coating or attachment to the apolipoprotein component of lipoproteins is common, including contrast-generating materials in the hydrophobic core of lipoproteins is rare. It is conceptually possible, however, to include many different hydrophobic materials in lipoprotein cores, as indicated by experiments where hydrophobic drugs have been incorporated into HDL for therapeutic purposes [23].

Cormode *et al.* recently reported a new generation of HDL-based contrast agents where, as well as modifying the phospholipid coating to include contrast-generating materials, hydrophobically coated inorganic nanocrystals were included in the particle core [24]. These nanocrystals were composed of gold (Au), iron oxide (IO) or cadmium selenide, also known as quantum dots (QDs). Gold, owing to its electron-dense nature, provides contrast for computed tomography (CT) [25], IO produces negative contrast (image darkening) in MRI [26] and QDs have excellent fluorescent properties [27]. The contrast agents formed from these different nanocrystals were termed Au-HDL, IO-HDL and QD-HDL. The doping of the phospholipid coating was adjusted according to the nanocrystal so that each particle gave contrast for MRI and fluorescence, while Au-HDL gave contrast for CT also (Figure 3A). Similar aspecific control particles were prepared with the polymer polyethyleneglycol (PEG) in the coating, without ApoA-I, and were termed Au-PEG, IO-PEG and QD-PEG.

The particle size, phospholipid coating and ApoA-I content were characterized and the nanocrystal HDL was found to be similar in these properties to native HDL. The affinity of these particles for macrophages was investigated via *in vitro* incubations. Significant uptake of the nanocrystal HDL in those cells was observed in CT, TEM, MRI and confocal microscopy imaging experiments (Figures 3B–F). The nanocrystal HDL was taken up at a greater rate than the pegylated control particles.

Consequently, the nanocrystal HDL contrast agents were applied in the ApoE^{-/-} mouse model of atherosclerosis. Substantial contrast was produced in the aortas of these mice 24 h post-injection of nanocrystal HDL, as evidenced by Figure 3G & H. By comparison, the pegylated control particles produced negligible contrast in the aortas of mice. In the case of Au-HDL, the aortas of the mice were excised and imaged using a microCT system (Figure 3I). The images produced showed bright spots in the aortas, which were not present in the aortas of control mice and were attributed to accumulation of the gold particles in these areas. Furthermore,

excised aortas were imaged using a fluorescence system, which confirmed the accumulation of the nanocrystal HDL agents in the aorta, for example, the fluorescent image of the aorta of a mouse injected with QD-HDL is shown in Figure 3J. Confocal microscopy performed on aortic sections showed these agents to be taken up largely in the macrophages also.

LDL contrast agents

Other lipoproteins have been adapted as contrast agents, with the majority of work focused on LDL [28]. From the mid-1980s, several groups labeled LDL with radioactive nuclei, such as ^{123}I , ^{111}In or $^{99\text{m}}\text{Tc}$, in order to investigate the behavior of LDL in patients [29–31]. LDL has been labeled with Mn^{2+} or Gd^{3+} to provide MRI contrast [32,33], and has also been labeled with the high relaxivity Gd-AAZTA-C17 lipid [34]. Such Gd-labeled LDL can be used as a tumor-targeted contrast agent owing to the high level of expression of the LDL receptor on the cells of some types of cancer [35]. Furthermore, Zheng *et al.* modified LDL with folic acid and a fluorophore [36]. This LDL contrast agent was used to specifically detect tumors that over-express the folate receptor via optical imaging techniques. This result is very significant, as it indicated that lipoprotein contrast agents could be redirected to targets other than their natural receptors.

The earlier adoption of LDL rather than HDL as a contrast agent probably stems from the early recognition of the atherogenic properties of LDL [29,37]. The atheroprotective qualities of HDL were discovered more recently [37] but are now a strong motivation for labeling and imaging HDL in patients. As a targeted contrast agent, LDL possesses some advantages over HDL, such as the higher payload of contrast-generating material LDL can carry. On the other hand, HDL can be more easily reconstituted than LDL [38] and is more suitable for use in patients with atherosclerosis, owing to its atheroprotective properties [37].

Critical discussion

While we have outlined many of the accomplishments in the area of HDL contrast agents, there are a number of questions that remain to be resolved in their use. A perennial problem that scientists face is whether performing an experiment alters the outcome. In the case of HDL, this particle has anti-inflammatory properties [39] and, when used to detect inflammation, there is potential that the baseline inflammation will be altered by the experiment. It is likely, however, that the alteration would be fairly consistent between patients and valuable information would still be provided on inflammation levels from a scoring system of the contrast produced in the aorta.

When using a molecular imaging contrast agent, it is necessary to perform postinjection imaging at a minimum of five-times the circulation half-life of the contrast agent, so that the signal from the contrast agent in the blood is sufficiently diminished. Molecular imaging contrast agents for MRI are largely nanoparticle based [40] and have circulation half-lives on the scale of hours. Consequently, MRI-based molecular imaging in the clinic will probably either necessitate two visits by patients, the first for baseline imaging and agent injection and the second for postinjection imaging, or a long wait between the first and second imaging sessions. In the experiments outlined above, the primary postinjection imaging timepoint used is 24 h and was arbitrarily chosen. However, in the case of P2A2-HDL, the short half-life would allow shorter postinjection timepoints (~3.3 h) to be used, so that only one hospital visit would be required – a comparable protocol to that of FDG-PET imaging [41]. In recent years, concern has arisen over the use of small-molecule gadolinium chelates in patients with impaired renal function, as a condition known as nephrogenic systemic fibrosis sometimes affects such patients who are given high doses of these contrast agents [42]. Nanoparticulate contrast agents that are too big for renal filtration (>6 nm) typically gather in the liver [43]. It is not known if Gd-loaded nanoparticles will result in any kind of toxicity in the liver; in some liver studies,

administration of high doses of GdCl_3 has exacerbated pre-existing disease [44], but in other studies it has had a therapeutic effect [45]. However, IO nanoparticles are already used in patients [46] and, therefore, IO-loaded HDL might be most easily accepted for use in the clinic.

Conclusion

Prior to these imaging studies, HDL had been proposed for use in drug delivery [24]. This suggested that its major dual advantages – easy reconstitution and ability to cross endothelium – could be adapted for imaging purposes. That this has been successfully accomplished is illustrated by the examples described above, as well as in the references cited in this article. Given the continuing efforts of imaging scientists, chemists and vascular biologists, working together in our own group and in other laboratories, the investigation of HDL as a versatile nanoplatform for imaging will continue to uncover exciting results for the foreseeable future.

Future perspective

Modified HDL is now well established to act as an imaging agent for macrophages in atherosclerosis in preclinical settings. We expect that this will be used to investigate the effect of therapy or other substances on macrophage density in animal models of atherosclerosis. Lipoproteins may also be rerouted to other disease targets as demonstrated by Zheng *et al.* [47]. Therefore HDL, modified with targeting ligands, may be used to detect other diseases, for example, cancer, as well as biological processes, such as angiogenesis. In addition, modifying HDL lipoproteins so that they can be tracked *in vivo* may allow new avenues to be opened for the investigation of their metabolism at the whole organism level.

Executive summary

Modification of HDL as a contrast agent

- HDL may be modified to include a variety of materials that generate contrast for medical imaging.

These materials include radioactive or paramagnetic elements, fluorophores and nanocrystals and, thus, HDL can produce contrast for nuclear imaging techniques, MRI, fluorescence imaging and computed tomography.

Use of HDL as a contrast agent

- Modified HDL has been used extensively in atherosclerotic mice to detect macrophage levels in plaques using MRI and has excellent potential for similar clinical applications.

Conclusion

- HDL is now well recognized as a platform for contrast agent development.

Future perspective

- Given the addition of appropriate targeting ligands, modified HDL could be used to image other disease markers as well as physiological and pathophysiological processes.

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Financial & competing interests disclosure

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Bibliography

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. Mulder WJM, Cormode DP, Hak S, Lobatto ME, Silvera S, Fayad ZA. Multimodality nanotracers for cardiovascular applications. *Nat. Clin. Pract. Cardiovasc. Med* 2008;5:S103–S111. [PubMed: 18641599]
2. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis through the arterial tree. *Arterioscl. Thromb* 1994;14:133–140. [PubMed: 8274468]
3. Fayad ZA, Fallon JT, Shinnar M, et al. Noninvasive *in vivo* high-resolution magnetic resonance imaging of atherosclerotic lesions in genetically engineered mice. *Circulation* 1998;98:1541–1547. [PubMed: 9769308] ▪ MRI visualization of the plaques of atherosclerotic mice.
4. Trogan E, Fayad ZA, Itskovich VV, et al. Serial studies of mouse atherosclerosis by *in vivo* magnetic resonance imaging detect lesion regression after correction of dyslipidemia. *Arterioscler. Thromb. Vasc. Biol* 2004;24:1714–1719. [PubMed: 15256400]
5. Choudhury RP, Fayad ZA, Aguinaldo JG, et al. Serial, noninvasive, *in vivo* magnetic resonance microscopy detects the development of atherosclerosis in apolipoprotein E-deficient mice and its progression by arterial wall remodeling. *J. Magn. Reson. Imaging* 2003;17:184–189. [PubMed: 12541225]
6. Shamir R, Johnson WJ, Morlock-Fitzpatrick K, et al. Pancreatic carboxyl ester lipase: a circulating enzyme that modifies normal and oxidized lipoproteins *in vitro*. *J. Clin. Invest* 1996;97:1696–1704. [PubMed: 8601635]
7. Lusis AJ. Atherosclerosis. *Nature* 2000;407:233–241. [PubMed: 11001066]
8. Shaish A, Keren G, Chouraqui P, Levkovitz H, Harats D. Imaging of aortic atherosclerotic lesions by ¹²⁵I-LDL, ¹²⁵I-oxidized-LDL, ¹²⁵I-HDL and ¹²⁵I-BSA. *Pathobiology* 2001;69:225–229. [PubMed: 12007282] ▪ HDL labeled with ¹²⁵I - the first report of HDL as a contrast agent.
9. Frias JC, Williams KJ, Fisher EA, Fayad ZA. Recombinant HDL-like nanoparticles: a specific contrast agent for MRI of atherosclerotic plaques. *J. Am. Chem. Soc* 2004;126:16316–16317. [PubMed: 15600321] ▪ First report of an MRI-active HDL contrast agent. It was demonstrated that these nanoparticles accumulated in macrophages in atherosclerotic plaques.
10. Frias JC, Ma Y, Williams KJ, Fayad ZA, Fisher EA. Properties of a versatile nanoparticle platform contrast agent to image and characterize atherosclerotic plaques by magnetic resonance imaging. *Nano. Lett* 2006;6:2220–2224. [PubMed: 17034087]
11. Briley-Saebo KC, Geninatti Crich S, Cormode DP, et al. High-relaxivity gadolinium-modified high-density lipoproteins as magnetic resonance imaging contrast agents. *J. Phys. Chem. B* 2009;113:6283–6289. [PubMed: 19361222]
12. Gianolio E, Giovenzana GB, Longo D, Longo I, Menegotto I, Aime S. Relaxometric and modelling studies of the binding of a lipophilic Gd-AAZTA complex to fatted and defatted human serum albumin. *Chem. Eur. J* 2007;13:5785–5797.
13. Chen W, Vucic E, Leupold E, et al. Incorporation of an apoE-derived lipopeptide in high-density lipoprotein MRI contrast agents for enhanced imaging of macrophages in atherosclerosis. *Contrast Media Mol. Imaging* 2008;3:233–242. [PubMed: 19072768]

14. Sauer I, Dunay IR, Weisgraber K, Bienert M, Dathe M. An apolipoprotein E-derived peptide mediates uptake of sterically stabilized liposomes into brain capillary endothelial cells. *Biochemistry* 2005;44:2021–2029. [PubMed: 15697227]
15. Navab M, Anantharamaiah GM, Reddy ST, Fogelman AM. Apolipoprotein A-I mimetic peptides and their role in atherosclerosis prevention. *Nat. Clin. Pract. Cardiovasc. Med* 2006;3:540–547. [PubMed: 16990839]
16. Frank PG PG, Marcel YL. Apolipoprotein A-I: structure–function relationships. *J. Lipid Res* 2000;41:853–872. [PubMed: 10828078]
17. Anantharamaiah GM, Jones JL, Brouillette CG, et al. Studies of synthetic peptide analogs of the amphipathic helix. *J. Biol. Chem* 1985;260:10248–10255. [PubMed: 4019510]
18. Davidson WS, Lund-Katz S, Johnson WJ, et al. The influence of apolipoprotein structure on the efflux of cellular free cholesterol to high density lipoprotein. *J. Biol. Chem* 1994;269:22975–22982. [PubMed: 8083197]
19. Anantharamaiah GM. Synthetic peptide analogs of apolipoproteins. *Method. Enzymol* 1986;128:627–647.
20. Tang C, Vaughan AM, Anantharamaiah GM, Oram JF. Janus kinase 2 modulates the lipid-removing but not protein-stabilizing interactions of amphipathic helices with ABCA1. *J. Lipid Res* 2006;47:107–114. [PubMed: 16210729]
21. Cormode DP, Briley-Saebo KC, Mulder WJM, et al. An apoA-I mimetic peptide HDL-based MRI contrast agent for atherosclerotic plaque composition detection. *Small* 2008;4:1437–1444. [PubMed: 18712752]
22. Cormode DP, Chandrasekar R, Delshad A, et al. Comparison of synthetic HDL contrast agents for MR imaging of atherosclerosis. *Bioconjugate Chem* 2009;20:937–943.
23. Rensen PCN, de Vroeh RLA, Kuiper J, Bijsterbosch MK, Biessen EAL, van Berkel TJC. Recombinant lipoproteins: lipoprotein-like lipid particles for drug targeting. *Adv. Drug Deliv. Rev* 2001;47:251–276. [PubMed: 11311995]
24. Cormode DP, Skajaa T, van Schooneveld MM, et al. Nanocrystal core high-density lipoproteins: a multimodal molecular imaging contrast agent platform. *Nano. Lett* 2008;8:3715–3723. [PubMed: 18939808]
25. Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new x-ray contrast agent. *Brit. J. Radiol* 2006;79:248–253. [PubMed: 16498039]
26. Bulte JWM, Kraitchman DL. Iron oxide MR contrast agents for molecular and cellular imaging. *NMR Biomed* 2004;17:484–499. [PubMed: 15526347]
27. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H. Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater* 2005;4:435–446. [PubMed: 15928695]
28. Frias JC, Lipinski MJ, Lipinski SE, Albelda MT. Modified lipoproteins as contrast agents for imaging of atherosclerosis. *Contrast Media Mol. Imaging* 2007;2:16–23. [PubMed: 17318917]
29. Sinzinger H, Bergmann H, Kaliman J, Angelberger P. Imaging of human atherosclerosis lesions using I-123 low-density-lipoprotein. *Eur. J. Nucl. Med* 1986;12:291–292. [PubMed: 3780773]
30. Rosen JM, Butler SP, Meinken GE, et al. Indium-111-labeled LDL: a potential agent for imaging atherosclerotic disease and lipoprotein biodistribution. *J. Nucl. Med* 1990;31:343–350. [PubMed: 2308006]
31. Ginsberg HN, Goldsmith SJ, Vallabajosula S. Noninvasive imaging of ^{99m}Tc-labeled low density lipoprotein uptake by tendon xanthomas in hypercholesterolemic patients. *Arteriosclerosis* 1990;10:256–262. [PubMed: 2317159]
32. Mitsumori LM, Ricks JL, Rosenfeld ME, Schmiedl UP, Yuan C. Development of a lipoprotein based molecular imaging MR contrast agent for the noninvasive detection of early atherosclerotic disease. *Int. J. Cardiovasc. Imaging* 2004;20:561–567. [PubMed: 15856643]
33. Li H, Gray BD, Corbin IR, et al. MR and fluorescent imaging of low-density lipoprotein receptors. *Acad. Radiol* 2004;11:1251–1259. [PubMed: 15561572]
34. Geninatti Crich S, Lanzardo S, Alberti D, et al. Magnetic resonance imaging detection of tumor cells by targeting low-density lipoprotein receptors with Gd-loaded low-density lipoprotein particles. *Neoplasia* 2007;9:1046–1056. [PubMed: 18084612]

35. Corbin IR, Li H, Chen J, et al. Low-density lipoprotein nanoparticles as magnetic resonance imaging contrast agents. *Neoplasia* 2006;8:488–498. [PubMed: 16820095]
36. Chen J, Corbin IR, Li H, Cao W, Glickson JD, Zheng G. Ligand conjugated low-density lipoprotein nanoparticles for enhanced optical cancer imaging *in vivo*. *J. Am. Chem. Soc* 2007;129:5798–5799. [PubMed: 17428054]
37. Gordon DJ, Rifkind BM. High-density lipoprotein – the clinical implications of recent studies. *N. Engl. J. Med* 1989;321:1311–1316. [PubMed: 2677733] •• Excellent review of the different methods available for the reconstitution of HDL.
38. Jonas A. Reconstitution of high-density lipoproteins. *Method. Enzymol* 1986;128:553–582.
39. Rye K-A, Barter PJ. Antiinflammatory actions of HDL: a new insight. *Arterioscler. Thromb. Vasc. Biol* 2008;28:1890–1891. [PubMed: 18946054]
40. Jaffer FA, Libby P, Weissleder R. Molecular imaging of cardiovascular disease. *Circulation* 2007;116:1052–1061. [PubMed: 17724271]
41. Rudd JHF, Myers KS, Bansilal S, et al. ¹⁸F-FDG PET: carotid, iliac and femoral uptake reproducibility, quantification methods, and recommendations. *J. Nucl. Med* 2008;49:871–878. [PubMed: 18483100]
42. Sieber MA, Pietsch H, Walter J, Haider W, Frenzel T, Weinmann HJ. A preclinical study to investigate the development of nephrogenic systemic fibrosis: a possible role for gadolinium-based contrast media. *Invest. Radiol* 2008;43:65–75. [PubMed: 18097279]
43. Choi HS, Liu W, Misra P, et al. Renal clearance of quantum dots. *Nat. Biotech* 2007;25:1165–1170.
44. Korolenko TA, Klishevich MS, Cherkanova MS, et al. *In vivo* effect of selective macrophage suppression on the development of intrahepatic cholestasis in mice. *Bull. Exp. Biol. Med* 2008;146:396–400. [PubMed: 19489305]
45. Ide M, Kuwamura M, Kotani T, Sawamoto O, Yamate J. Effects of gadolinium chloride (GdCl₃) on the appearance of macrophage populations and fibrogenesis in thioacetamide-induced rat hepatic lesions. *J. Comp. Pathol* 2005;133:92–102. [PubMed: 15964588]
46. Trivedi RA, U-King-Im JM, Graves MJ, et al. *In vivo* detection of macrophages in human carotid atheroma – temporal dependence of ultrasmall superparamagnetic particles of iron oxide-enhanced MRI. *Stroke* 2004;35:1631–1635. [PubMed: 15166394]
47. Zheng G, Chen J, Li H, Glickson JD. Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. *Proc. Natl Acad. Sci. USA* 2005;102:17757–17762. [PubMed: 16306263] •• First paper to show that lipoprotein-based contrast agents can be redirected to targets other than their natural receptors.

Figure 1. *In vivo* T1-weighted MRI at different time points (pre- and post-injection of contrast agent at 0.5, 24 and 48 h) and dosages of gadolinium. White arrows point to the abdominal aorta; the insets denote a magnification of the aorta region. Parts of this figure are reproduced with permission from [9].

Figure 2. Imaging of atherosclerosis with synthetic, Gd-labeled HDL

(A) Edmundson wheel representation of the ApoA-I mimicking peptide 18A. The peptide used in this study was 37pA, whose sequence is 18A-P-18A. (B) Schematic representation of 37pA, a synthetic, HDL-based contrast agent. (C) 37pA-Gd produced a similar degree of macrophage cholesterol efflux as native HDL. (D) T₁-weighted MRI of the aorta (arrows) of an ApoE^{-/-} mouse pre- and 24 h post-injection with 37pA-Gd. (E) Confocal microscopy of an excised aorta of an ApoE^{-/-} mouse injected 24 h previously with 37pA-Gd. Blue: nuclei, red: 37pA-Gd and green: macrophages. The extensive yellow coloration is due to colocalization of 37pA and macrophages.

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Figure 3. Nanocrystal core HDL allows imaging of atherosclerosis using MRI, computed tomography and fluorescence imaging techniques
 (A) Nanocrystal core HDL. (B) Transmission electron microscopy (TEM) image of pellets (in paraformaldehyde) of macrophage cells that had been incubated with either Au-HDL or Au-PEG. (C) TEM image of Au-HDL uptake in a macrophage cell. (D) T₂-weighted MRI of pellets of macrophage cells that had been incubated with IO-HDL, IO-PEG or with media only. Confocal images of cells incubated with (E) QD-HDL or (F) QD-PEG. T₂*-weighted MR's of the aorta of an ApoE^{-/-} mouse (G) preinjection and (H) 24 h postinjection of IO-HDL. (I) Micro-computed tomography image of an excised aorta of an ApoE^{-/-} mouse injected 24 h previously with Au-HDL. The bright spots indicate uptake of Au-HDL in atherosclerotic

tissue. **(J)** Fluorescence image of an aorta excised from an ApoE^{-/-} mouse that had been injected 24 h previously with QD-HDL.

AU: Silver; IO: Iron oxide; QD: Quantum dot.

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