Exploration and application of nanomedicine in atherosclerotic disease
Lobatto, M.E.

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Chapter 5

In vivo PET imaging of HDL in multiple atherosclerosis models

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Abstract

Objectives: The goal of this study was to develop and validate a noninvasive imaging tool to visualize the in vivo behavior of high-density lipoprotein (HDL) by using positron emission tomography (PET), with an emphasis on its plaque-targeting abilities.

Background: HDL is a natural nanoparticle that interacts with atherosclerotic plaque macrophages to facilitate reverse cholesterol transport. HDL-cholesterol concentration in blood is inversely associated with risk of coronary heart disease and remains one of the strongest independent predictors of incident cardiovascular events.

Methods: Discoidal HDL nanoparticles were prepared by reconstitution of its components apolipoprotein A-I (APOA1) and the phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine. For radiolabeling with zirconium-89 ($^{89}$Zr), the chelator deferoxamine B was introduced by conjugation to APOA1 or as a phospholipid-chelator (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-deferoxamine B). Biodistribution and plaque targeting of radiolabeled HDL was studied in established murine, rabbit and porcine atherosclerosis models by using PET combined with computed tomography (PET/CT) or PET combined with magnetic resonance imaging (PET/MRI). Ex vivo validation was conducted by radioactivity counting, autoradiography and near infrared fluorescence imaging. Flow cytometric assessment of cellular specificity in different tissues was performed in the murine model.

Results: We observed distinct pharmacokinetic profiles for the two $^{89}$Zr-HDL nanoparticles. Both APOA1- and phospholipid-labeled HDL accumulated in the vessel wall as well as kidneys, liver, spleen and bone marrow with some marked quantitative differences in radioactivity uptake values. Radioactivity concentrations in rabbit atherosclerotic aortas were 3- to 4-fold higher than in control animals at 5 days’ post-injection for both $^{89}$Zr-HDL nanoparticles. In the porcine model, increased accumulation of radioactivity was observed by using in vivo PET imaging. Irrespective of the radiolabel’s location, HDL nanoparticles were able to preferentially target plaque macrophages and monocytes.

Conclusions: $^{89}$Zr labeling of HDL allows study of its in vivo behavior by non-invasive PET imaging, including visualization of its accumulation in advanced atherosclerotic lesions. The different labeling strategies provide insight on the pharmacokinetics, biodistribution and metabolism of HDL’s main components (i.e. phospholipids, APOA1).
**Introduction**

Atherosclerosis is a systemic inflammatory disorder that underlies cardiovascular disease\(^1\). Lipid deposition and immune cell infiltration drive development of atherosclerotic plaque\(^2\), which can eventually rupture\(^3\), potentially causing atherothrombosis and ensuing acute coronary events\(^4\).

High-density lipoprotein (HDL), a natural nanoparticle mainly composed of phospholipids, cholesterol and cholesteryl esters, and apolipoprotein A-I (APOA1), is involved in the process of reverse cholesterol transport\(^5\). HDL and APOA1 have demonstrated atheroprotective properties\(^6-8\). The principal mechanism whereby HDL exerts this protective effect is generally ascribed to promoting cholesterol efflux from macrophages in plaques and transporting it to the liver for excretion, although other anti-atherogenic properties have been reported\(^9\). Cholesterol efflux is mediated by several membrane receptors abundantly expressed on macrophages to which APOA1 and HDL bind\(^10,11\).

The study of HDL can be modernized by the integration of noninvasive imaging. Capitalizing on its properties, our group pioneered the use of HDL magnetic resonance imaging (MRI)\(^12,13\). However, to noninvasively study the pharmacokinetics, trafficking, and metabolism of HDL, a quantitative and highly sensitive modality is warranted. Recent advances in labeling technology now allow positron emission tomography (PET) radiotracer imaging at unprecedented high-sensitivity down to the picomolar range, at limitless tissue penetration, in a so-called “hot spot fashion”\(^14\). Tracing of HDL requires long-lived radioisotopes because, upon intravenous administration, it circulates for extended periods of time. Recently, we have developed modular zirconium-89 (\(^{89}\text{Zr}\))-labeling methods for lipid-based nanoparticles\(^15,16\). The physical half-life of \(^{89}\text{Zr} (78.4 \text{ h})\) makes it an ideal radiolabel for such long-circulating materials\(^15\), and provides an opportunity to study the *in vivo* behavior of the HDL nanoparticles. Through extensive studies involving a unique set-up combining PET/computed tomography (CT) and PET/MRI in murine, rabbit and porcine atherosclerosis models, the present paper illustrates that \(^{89}\text{Zr} labeling is a valuable tool to non-invasively assess pharmacokinetics, distribution, metabolism and turnover of HDL’s main components.

**Results**

**Radiolabeling of HDL nanoparticles.** The composition, dynamic light scattering-measured size and size exclusion retention time for all HDL nanoparticles used in this study are summarized in table S1. To incorporate the radioactive label (\(^{89}\text{Zr}\)), we either conjugated deferoxamine B to APOA1 via its attachment to lysine residues, or included the phospholipid chelator 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-deferoxamine B in the formulation of the particles. Reactions of these deferoxamine B-bearing nanoparticles with \(^{89}\text{Zr}-\text{oxalate generated }^{89}\text{Zr-APOA1-labeled HDL (}\(^{89}\text{Zr-AI-HDL}) or }^{89}\text{Zr-phospholipid-labeled HDL (}\(^{89}\text{Zr-PL-HDL, figure 1A}), at high radiochemical yields were high (96 ± 2 \([n = 5]\) and 81 ± 10 % \([n = 7]\), respectively), and radiochemical purities of >98%. Radiolabeling did not impact the size of HDL.

**Cellular targets of HDL nanoparticles.** In addition, fluorescent, nonradioactive analogs of the 2 \(^{89}\text{Zr}-labeled HDL nanoparticles were prepared to investigate their cellular targets in different tissues by using flow cytometry (figures 1B and S1). These particles were prepared in a manner identical to that of their radioactive counterparts, but contained the dye DiR (table S1) and non-radioactive Zr. In Apoe\(^/-\) mice, flow cytometric analyses revealed little targeting differences between the 2 Zr-labeled nanoparticles in blood, spleen and aorta at 24 h post-injection (figure 1C). In atherosclerotic aortas, macrophages and monocytes were preferentially targeted over neutrophils (4.5- and 3.1-fold greater DiR uptake, respectively) and lineage-positive cells (290- and 220-fold greater DiR uptake), whereas mostly macrophages were targeted in the spleen. In blood, dendritic cells and monocytes were targeted to nearly the same extent.
Figure 1. $^{89}$Zr-HDL nanoparticles and their cellular targets in a mouse atherosclerosis model. (A) Schematic of zirconium-89 apolipoprotein A1-labeled high-density lipoprotein ($^{89}$Zr-AI-HDL) and zirconium-89 phospholipid-labeled HDL ($^{89}$Zr-PL-HDL). (B) Gating procedure and cell targets of Zr-HDL nanoparticles in mouse atherosclerotic aortas. Nonradioactive fluorescent DiR-labeled analogues were used to determine cell-targeting preference in the aortas of ApoE-/— mice (18 weeks on a high-fat diet [HFD]) by using flow cytometry. (C) Quantification of DiR concentrations as mean fluorescence intensity (MFI) in different cell types in the aorta, spleen and blood of ApoE-/— mice (18 weeks on HFD).

Pharmacokinetics and biodistribution of $^{89}$Zr-HDL nanoparticles in mice. Blood radioactivity clearance in ApoE-/— and wild-type mice was investigated (figure 2A). The weighted half-lives for $^{89}$Zr-AI-HDL-associated radioactivity in blood were 1.4 and 2.0 h in atherosclerotic and wild-type mice, respectively, whereas for $^{89}$Zr-PL-HDL (figure 2A, bottom) these values were 2.8 and 1.1 h. Radioactivity distribution at 24 and 48 h was also measured for selected tissues. Figure 2B illustrates a comparison of radioactivity uptake values for both nanotracers at 24 h post-injection, whose target organs were the same. For both $^{89}$Zr-AI-HDL and $^{89}$Zr-PL-HDL kidneys were the main radioactivity accumulation site, although with marked quantitative differences. No statistically significant differences were found in tissue uptakes for $^{89}$Zr-AI-HDL between diseased and control animals. However, when whole organ radioactivity accumulation was considered, uptake in kidneys, liver and spleen was significantly higher for atherosclerotic mice (figure S2A). For $^{89}$Zr-PL-HDL, kidney and liver uptakes were significantly higher for control animals. At 48 h post-injection (figure S2B and C), kidneys were also the main activity accumulation site for both nanoparticles. For $^{89}$Zr-AI-HDL, uptakes were as high as 80 %ID/g (i.e. the percent injected dose per gram).
**Micro-PET/CT imaging studies in mice.** Noninvasive visualization of $^{89}$Zr-HDL nanoparticles' biodistribution in mice was investigated at 24 (figure 2C) and 48 h (figure S2B) after intravenous administration. For $^{89}$Zr-AI-HDL images were clearly dominated by strong signal from the kidneys whereas for $^{89}$Zr-PL-HDL liver and kidneys were the preferred accumulation sites. PET-derived uptake values were in good agreement with the *ex vivo* results (figure S2D).

![Figure 2](image)

**Figure 2.** $^{89}$Zr-HDL pharmacokinetics and PET/CT imaging in mice. (A) Blood time-activity curves for $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) in Apoe$^{-/-}$ (18 weeks on HFD) and wild-type B6 mice ($n = 3$ per group). (B) Radioactivity distribution in selected tissues for $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) at 24 h post-injection ($n ≥ 4$ per group). (C) positron emission tomography (PET)/computed tomography (CT) imaging of $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) at 24 h post-injection in Apoe$^{-/-}$ (18 weeks on HFD), showing CT images only (left), PET/CT fusion images (middle; scale bar indicates injected dose per gram (%ID/g)) and 3-dimensional rendering of the PET/CT fusion image (right). $^*$ $P < 0.05$. Ki = kidney; Li = liver; Lu = lungs; Mu = muscle; Sp = spleen; other abbreviations as in figure 1.

**Plaque targeting of $^{89}$Zr-HDL nanoparticles in mice.** Increased radioactivity accumulation in whole aortas of Apoe$^{-/-}$ mice was found for both nanoparticles at 24 h. For $^{89}$Zr-AI-HDL, the difference was significantly higher when only the aortic root was considered (figure 3A). At 48 h, a significantly higher accumulation in atherosclerotic aortas was found for $^{89}$Zr-PL-HDL only (0.019 ± 0.04 %ID [n = 6] vs. 0.013 ± 0.03 %ID [n = 4] for control animals, $P = 0.02$). In autoradiographic analysis, higher deposition of radioactivity was observed in atherosclerotic aortas (figure 3B), especially in aortic roots. Increased plaque accumulation of the fluorescent analogs of $^{89}$Zr-HDL nanoparticles (DiR@Zr-AI-HDL and DiR@Zr-PL-HDL) was also observed in atherosclerotic aortas by using near infrared fluorescence (NIRF) imaging. Good correlations were found for both $^{89}$Zr-AI-HDL ($\rho = 0.80$) and $^{89}$Zr-PL-HDL ($\rho = 0.72$) between total radioactivity accumulation and total radiant efficiency of their fluorescent analogs at 24 h post-injection (figure 3C).
Figure 3. Plaque targeting of $^{89}$Zr-HDL nanoparticles in $\text{Apoe}^{-/-}$ mice. (A) Comparison between radioactivity content and near-infrared fluorescence (NIRF) intensity (total radiant efficiency in μW/cm$^2$) in the aortic roots of $\text{Apoe}^{-/-}$ (18 weeks on HFD) and wild-type B6 mice at 24 h post-injection of $^{89}$Zr-Al-HDL and DiR@Zr-Al-HDL (top) and $^{89}$Zr-PL-HDL and DiR@Zr-PL-HDL (bottom) ($n \geq 4$ per group). (B) Autoradiography and NIRF images of whole aortas of $\text{Apoe}^{-/-}$ (18 weeks on HFD) and wild-type B6 mice at 24 h post-injection of $^{89}$Zr-Al-HDL and DiR@Zr-Al-HDL (top) and $^{89}$Zr-PL-HDL and DiR@Zr-PL-HDL (bottom). (C) Correlation between radioactivity content and NIRF intensity (total radiant efficiency, in μW/cm$^2$) in the aortic roots of $\text{Apoe}^{-/-}$ (18 weeks on HFD) and wild-type B6 mice at 24 h post-injection of $^{89}$Zr-Al-HDL and DiR@Zr-Al-HDL (top) and $^{89}$Zr-PL-HDL and DiR@Zr-PL-HDL (bottom) ($n \geq 4$ per group). * $P < 0.05$.

Pharmacokinetics and biodistribution of $^{89}$Zr-HDL nanoparticles in rabbits. Similarly to the murine model, radioactivity clearance was slower for $^{89}$Zr-Al-HDL (figure 4A), whose half-lives were 1.13 and 1.05 days in control and atherosclerotic animals, respectively. For $^{89}$Zr-PL-HDL (figure 4A), clearance was slower in animals with atherosclerosis (0.44 vs. 0.34 day for control animals). Radioactivity distribution in selected tissues was measured at 5 days post-injection (figure 4B). Kidneys exhibited the highest uptake values for both nanoparticles, although they were 3- to 4-fold higher for $^{89}$Zr-Al-HDL. Spleen and liver uptakes were around 0.1 %ID/g for the two nanoparticles.

Imaging studies in rabbits. The $^{89}$Zr-HDL nanoparticles were evaluated in rabbits by using in vivo PET imaging on a clinical PET/CT scanner. Representative PET/CT fusion images of the abdominal region at three different time points can be seen in figure 4C for both nanoparticles. In addition, a unique clinical PET/MRI system to investigate the in vivo behavior of the nanoparticles in the same animals and time points for direct comparison. Figure 5A displays representative PET/MRI fusion images of the same animals shown in figure 4C. The PET images obtained with both scanners were almost identical (online video 1). More importantly, there was a strong correlation between the standardized uptake values (SUVs) measured from both systems (figure 5B) and also excellent agreement between the values (figure 5C), with an intraclass correlation coefficient of 0.99 (95% confidence interval: 0.98 to 0.99). Time-activity curves based on data obtained from PET/CT and PET/MRI analysis are shown in figure S3 and S4, respectively. Initially, within the first hour after intravenous administration,
images were dominated by a strong blood pool signal for both $^{89}$Zr-AI-HDL and $^{89}$Zr-PL-HDL (figure 4C and figure 5A), as well as high signal from the liver, spleen and kidneys. Blood radioactivity half-lives derived from PET/CT SUVs measured in the cardiac chambers were shorter than those obtained by blood sampling for both $^{89}$Zr-AI-HDL (0.55 and 0.56 days for diseased animals control animals, respectively) and $^{89}$Zr-PL-HDL (0.27 and 0.31 days for diseased animals and control animals). At later time points, $^{89}$Zr-AI-HDL images showed very high radioactivity accumulation in kidneys, which was significantly higher in atherosclerotic animals at all time points investigated (figure S3A and S4A). For $^{89}$Zr-PL-HDL, strong gall bladder signals with SUVs as high as 20 g/mL (figure 4C) were observed at 1 and 2 days’ post-injection, which was accompanied by high radioactivity accumulation in the stomach and intestines. Intense signals from the kidneys were also observed at all time points for $^{89}$Zr-PL-HDL. In both cases, bone radioactivity accumulation was also detected over the five-day period (SUVs around 5 g/mL). Ex vivo analysis proved that this was mostly due to bone marrow uptake, as opposed to mineral bone. Bone marrow uptake was higher in animals with atherosclerosis for $^{89}$Zr-AI-HDL (0.17 ± 0.03 vs. 0.13 ± 0.01 %ID/g for control animals); for $^{89}$Zr-PL-HDL values were similar (0.039 ± 0.010 and 0.050 ± 0.002 %ID/g for rabbits with atherosclerosis and control animals). PET-measured SUVs were in good agreement with SUVs determined according to ex vivo gamma counting (figure S5), although they were typically lower.

Increased accumulation of activity was found at day 5 post-injection for both probes in atherosclerotic aortas compared with control aortas according to PET/CT imaging (figure 6A). The difference was statistically significant for $^{89}$Zr-PL-HDL ($P = 0.03$). For $^{89}$Zr-AI-HDL at 1, 2 and 3 days post-injection the high blood pool signal dominated the measurements, as evidenced by the similar blood and aorta ratios between SUVs in atherosclerotic and control rabbits (figure S6A). We found a very similar increased accumulation in atherosclerotic aortas for either $^{89}$Zr-AI-HDL (0.40 ± 0.08 vs. 0.34 ± 0.10 g/mL) or $^{89}$Zr-PL-HDL (0.24 ± 0.13 vs. 0.15 ± 0.08 g/mL) at day 5 post-injection. At earlier time points, values were similar for both nanoparticles in animals with atherosclerosis and in control animals.

Figure 4. $^{89}$Zr-HDL pharmacokinetics and PET/CT imaging in rabbits. (A) Blood time-activity curves for $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) in rabbits with atherosclerosis and wild-type control animals (n ≥ 4 per group). (B) Radioactivity distribution in selected tissues for $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) at 5 days post-injection (n ≥ 4 per group). (C) PET/CT fusion images of $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) at 1 h, 2 days’ and 5 days’ p.i in rabbits with atherosclerosis. * $P < 0.05$. GB = gall bladder; other abbreviations as in figures 1 and 2.
**Figure 5.** PET/MRI imaging of $^{89}$Zr-HDL in rabbits. A) PET/magnetic resonance imaging (MRI) fusion images of $^{89}$Zr-Al-HDL (top) and $^{89}$Zr-PL-HDL (bottom) at 1 h, 2 days’ and 5 days’ post-injection in rabbits with atherosclerosis. (B) Correlation between SUVs determined by using PET/MRI and PET/CT imaging for the same tissue and time point (n = 295). (C) Bland-Altman plot of the difference ($\text{SUV}_{\text{PET/CT}} - \text{SUV}_{\text{PET/MRI}}$) and average SUVs obtained from the PET/CT and PET/MRI systems for the same tissue and time point (n = 295).

**Plaque targeting of $^{89}$Zr-HDL nanoparticles in rabbits.** Radioactivity concentration was significantly higher in atherosclerotic aortas for both $^{89}$Zr-Al-HDL ($P = 0.03$) and $^{89}$Zr-PL-HDL ($P = 0.03$) as determined by gamma counting at 5 days’ post-injection (figure 6A). Autoradiography of explanted aortas revealed a patchy distribution of radioactivity in atherosclerotic aortas, illustrating preferential accumulation in lesions (figure 6B). Analysis according to NIRF imaging found increased accumulation of Cy5.5-HDL in atherosclerotic aortas. A good correlation was found between total radiant efficiency and radioactivity concentration (figures 6C and S6B). Interestingly, the correlation between radioactivity concentration and Cy7-albumin NIRF intensity was weak (figure S6C), suggesting permeability may not be an HDL accumulation determinant.
Figure 6. Plaque targeting of $^{89}$Zr-HDL nanoparticles in rabbits with atherosclerosis. (A) Comparison between SUVs measured from PET/CT images and ex vivo radioactivity concentration in aortas of rabbits with atherosclerosis and wild-type control animals at 5 days' post-injection of $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) (n = 4 per group). (B) Autoradiography and NIRF images of whole aortas from rabbits with atherosclerosis and wild-type control animals at 5 days' post-administration of $^{89}$Zr-AI-HDL and Cy5.5-HDL (top) and $^{89}$Zr-PL-HDL and Cy5.5-HDL (bottom). (C) Comparison between NIRF intensity (total radiant efficiency, in μW/cm$^2$) and radioactivity concentration in aortas of rabbits with atherosclerosis and wild-type control animals at 5 days' post administration of $^{89}$Zr-AI-HDL and Cy5.5-HDL (top) and $^{89}$Zr-PL-HDL and Cy5.5-HDL (bottom) (n = 4 per group). * $P < 0.05$.

Oxidized HDL imaging experiments in atherosclerotic rabbits. Blood radioactivity clearance for radiolabeled $^{89}$Zr-APOA1 labeled oxidized HDL ($^{89}$Zr-AI-HDL$^{Ox}$) nanoparticles was significantly faster than for $^{89}$Zr-AI-HDL in both animals with atherosclerosis and in the control animals (figure S7A), and its half-life was 3 times as short ($t_{1/2} = 0.33$ day). Radioactivity distribution, however, showed a similar pattern at day 5 post-injection. $^{89}$Zr-AI-HDL$^{Ox}$ uptake values were significantly lower in liver and lungs compared to $^{89}$Zr-AI-HDL in both atherosclerotic and control animals, and in the spleen compared to $^{89}$Zr-AI-HDL in diseased animals. Images from PET/CT (figure S7C) and PET/MR analysis revealed high accumulation in kidneys at all time points, with SUVs as high as 48 g/mL (1.0 %ID/g). These values were significantly higher than those measured for $^{89}$Zr-AI-HDL in controls animals and rabbits with atherosclerosis (figure S7D). PET-quantified SUVs were qualitatively similar to the values obtained after the day 5 scan by using ex vivo gamma counting (figure S7D). Interestingly, radioactivity accumulation in atherosclerotic aortas for $^{89}$Zr-AI-HDL$^{Ox}$ was significantly lower than for non-oxidized $^{89}$Zr-AI-HDL (figure S7E).

Imaging studies in pigs. Three pigs were imaged at 48 h after administration of $^{89}$Zr-PL-HDL. A whole-body PET maximum intensity projection image obtained on the clinical PET/CT scanner can be seen in figure 7A. Strong signals were observed in kidneys, liver and bone, as well as in the intestines. High radioactivity accumulation in the femoral arteries could be clearly observed in vivo (figure 7B). Radioactivity concentration in these vessels was higher than in non-injured internal iliac arteries, which served as controls (figure 7B). Radioactivity distribution in selected organs determined by gamma counting after the 48 h scan is shown in figure 7C, and mirrors the PET imaging observations. The increased signal in the femoral arteries was due to accumulation in lesions, as corroborated by ex vivo autoradiography analysis (figure 7D).
Figure 7. PET imaging and plaque targeting of $^{89}$Zr-PL-HDL in a porcine model of atherosclerosis. (A) Whole-body maximum intensity projection PET image of a pig injected with $^{89}$Zr-PL-HDL at 48 h post-injection (arrows indicating atherosclerotic femoral arteries). (B) Axial PET/CT image showing high radioactivity accumulation in lesions at 48 h post-injection (left), and quantification of radioactivity concentrations (right) in internal iliac (I.I., chosen as control) and femoral arteries (Fe) ($n = 3$). (C) Radioactivity distribution in selected tissues for $^{89}$Zr-Al-HDL at 48 h post-injection determined by gamma counting ($n = 3$). (D) Autoradiography of femoral arteries (left) and quantification of radioactivity concentration (counts per unit area) in non-injured tissue (N.I.) and lesions (Le) determined from the autoradiography analysis (right) ($n = 3$).
Discussion

In the present study, we developed and validated a noninvasive quantifiable PET imaging tool to study the in vivo behavior of HDL in multiple atherosclerosis models. Using nonradioactive fluorescent analogs of the $^{89}$Zr-labeled nanoparticles we found that both preferentially targeted macrophages in the atherosclerotic aortas of $\text{Apoe}^{-/-}$ mice, in line with our previous studies. This finding also suggests that the modifications were well tolerated and did not affect the ability of HDL ability to interact with plaque macrophages.

Radioactivity clearance followed similar patterns in both mice and rabbits, exhibiting longer half-lives for $^{89}$Zr-AI-HDL (apolipoprotein-labeled) in healthy control animals and for $^{89}$Zr-PL-HDL (phospholipid-labeled) in atherosclerotic animals. $^{89}$Zr-AI-HDL's longer half-life compared to $^{89}$Zr-PL-HDL in control animals may be explained by the low net internalization rate and recycling of the protein. However, the slower clearance of $^{89}$Zr-PL-HDL in animals with atherosclerosis, especially in the $\text{Apoe}^{-/-}$ mouse model, may be due to the elevated lipoprotein blood pool concentration and impaired lipid metabolism and clearance.

Modification of APOA1 can affect its blood circulation time as reported for radioiodination, which has been frequently used to study the pharmacokinetics and metabolism of the protein and of HDL. This labeling strategy is convenient and results in iodination of tyrosine residues as well as faster clearance than the unmodified protein, which is most likely due to rapid deiodination by deiodinases. Therefore, label stability and kinetics play an important role when designing an imaging tool. Importantly, using our approach, the radioactivity blood half-life for $^{89}$Zr-AI-HDL in rabbits is in close agreement with the value obtained by radioimmunometric assay using unmodified human APOA1 in the same animal, implying that the biological function is retained.

Our imaging studies show the same patterns in radioactivity distribution for the 3 animal models used; the kidneys were the main site of accumulation for both $^{89}$Zr-AI-HDL and $^{89}$Zr-PL-HDL. It is well established that the kidney is a major catabolism site for APOA1 and certain HDL subclasses. Quantitative analysis revealed a significantly higher kidney uptake in atherosclerotic rabbits at all time points for $^{89}$Zr-AI-HDL. The SUVs showed little variation over time, suggesting a steady-state situation. More importantly, the higher kidney uptakes found for $^{89}$Zr-AI-HDL in rabbits with atherosclerosis were accompanied by lower blood radioactivity concentrations; indeed, a higher glomerular filtration rate has been related to lower HDL and APOA1 blood levels in humans without kidney disease and to subclinical cardiovascular disease in non-diabetic subjects.

Although $^{89}$Zr-AI-HDL showed very high uptake in the kidneys, the radioactivity for $^{89}$Zr-PL-HDL was more evenly distributed between the kidneys, liver and spleen and, at later time points, the digestive system. Initially, very high uptakes were found in the gall bladder (both for control animals and animals with atherosclerosis), suggesting incorporation of the radiolabeled phospholipid to the bile and subsequent excretion. The detection of radioactivity in the stomach can be explained by the presence of the radioactive compound in the ceotropes (nutritious stools resulting from gut bacteria digestion) that rabbits selectively ingest.

Ex vivo analysis of rabbit bone samples revealed that most of this uptake originated from the bone marrow and that, for $^{89}$Zr-AI-HDL, this uptake was higher in animals with atherosclerosis. This may reflect the interaction of $^{89}$Zr-HDL nanoparticles with hematopoietic stem and multipotent progenitor cells, which overexpress the ABCG1 transporter involved in HDL-mediated cholesterol efflux.

Interestingly, blood radioactivity clearance for oxidized HDL nanoparticles ($^{89}$Zr-AI-HDL) in atherosclerotic animals was significantly faster than its nonoxidized counterpart in both control and diseased animals and was paralleled by a significantly increased kidney radioactivity accumulation. The shorter blood half-life of $^{89}$Zr-AI-HDL is probably the main reason why its accumulation in the plaque is significantly decreased when compared to nonoxidized $^{89}$Zr-AI-HDL, but impaired interaction with its receptors could also be partly responsible. These results are in agreement with a recent study using myeloperoxidase-oxidized APOA1. As a proof of principle, we tested 1 of our $^{89}$Zr-labeled nanoparticles, namely $^{89}$Zr-PL-HDL, in a porcine model of atherosclerosis. The target organs were the same (mainly kidneys and liver), with SUVs comparable to those observed in the rabbit studies. Atherosclerotic lesions were clearly visible at 48 h post administration despite the blood pool signal, suggesting that vessel...
wall inflammation can be imaged with HDL-PET in severe cases. In addition to PET/CT imaging, we also performed extensive PET/MRI studies in the rabbit model. Importantly, the PET/MRI data were in excellent agreement with the PET/CT data. Integrative PET/MRI has only recently become available and allows direct vessel (morphology) visualization, without administration of an additional agent to delineate the vasculature. To the best of our knowledge, this is the first time PET/MRI has been applied to monitor nanoparticle accumulation in the atherosclerotic vessel wall.

**Study limitations.** HDL, an aggregate of lipids and protein, inherently is a dynamic system, constantly converting into particles of different shapes and sizes by exchanging components with cells and other lipoproteins. Since APOA1 is the protein component that dictates HDL’s biological function, $^{89}$Zr-AI-HDL will primarily allow apolipoprotein tracking. Radioactivity biodistribution for $^{89}$Zr-PL-HDL, on the other hand, will be the result of complex exchange processes, as a fraction of the radiolabeled phospholipids will be exchanged with other plasma proteins. A third labeling strategy that we did not develop, i.e., the inclusion of a lipophilic $^{89}$Zr label, could shed light on lipid transportation throughout the body. PET imaging of the vessel wall has inherent limitations, mainly related to the limited spatial resolution. Therefore, reliable quantification of vessel wall uptake requires a nearly complete radioactivity clearance from circulation, which takes days for $^{89}$Zr-PL-HDL and even longer for $^{89}$Zr-AI-HDL.

**Conclusions**

$^{89}$Zr labeling allows study of the *in vivo* behavior of HDL by using PET combined with CT or MRI. This tool allows the noninvasive evaluation of HDL and could be of great value to study its metabolism and trafficking in preclinical and clinical settings. Ultimately, in the context of advanced clinical atherosclerosis, this noninvasive imaging tool is ideal for identifying patients amenable to HDL therapy and subsequent treatment monitoring in a theranostic fashion.
Methods

Materials. Phospholipids were purchased from Avanti Polar Lipids, and 1-(4-Isothiocyanatophenyl)-3,6,17-dihydroxy-7,10,18,21-tetraoxo-27-[N-acetylhydroxy-xylamoino]-6,11,17,22-tetraazaheptacicosanethiourea (DFO-p-NCS), from Macrocyclics. The dyes 1,1'-dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine Iodide (DiR), Cy7 mono-NHS ester and sulfo-Cy5.5 mono-NHS ester were acquired from Life Technologies, Kerafast and GE Healthcare Life Sciences, respectively. APOA1 from human plasma was purchased from Athens Research and Technology. Antibodies for flow cytometry were acquired from ebiosciences, Biolegend, and BD Bioscience. DSPE-DFO was prepared as previously reported. All other reagents were acquired from Sigma-Aldrich.

Animals. Female ApoE−/− and wild type C57BL/6j mice were purchased from Jackson Laboratories (Sacramento, CA). Male CRL SPF White New Zealand rabbits and Male familial hypercholesterolemia (FHC) pigs were acquired from Charles River Laboratories and University of Wisconsin, respectively. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees of Mount Sinai, Memorial Sloan Kettering Cancer Center and/or Skirball Center for Cardiovascular Research, and followed National Institutes of Health guidelines for animal welfare.

Mouse model of atherosclerosis. Female ApoE−/− mice (B6.129P2-Apoemtm1Unc/J, 4-6 weeks old) were fed a high fat diet (Harlan Teklad TD.88137, 42 % calories from fat) for 18 weeks. Under these conditions, this animal model develops atherosclerotic lesions due to high LDL cholesterol concentrations in blood resulting from their lack of apolipoprotein E.

Rabbit model of atherosclerosis. Male CRL SPF White New Zealand rabbits (2.5-3 months old) underwent double balloon injury of the thoracic and abdominal aorta to induce atherosclerosis. Denudation was performed by introducing a 4F-Fogarty embolectomy catheter (Edwards Lifesciences, Irvine, CA) through the femoral artery and inflating the balloon to 2 atm under fluoroscopic guidance. The procedure was repeated on the contralateral extremity 4 weeks later. Surgery was performed under anesthesia with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg). To further accelerate plaque progression, animals were fed a high cholesterol diet (Research diets, New Brunswick, NJ) enriched initially with 0.3% and subsequently 0.15% until study termination approximately 4 months later. Injured rabbits were imaged by intravascular ultrasonography (IVUS) weekly for 2 months and the procedure was repeated on the contralateral extremity 4 weeks later.

Preparation of reconstituted HDL (rHDL) and radiosynthesis of 89Zr-HDL nanoparticles. A detailed description of the formulation and synthetic procedures can be found elsewhere. Briefly, reconstituted HDL (rHDL) was prepared at a DMPC-to-APOA1 weight ratio of 2.5:1. Ready-to-label nanoparticles were prepared by conjugation of DFO-p-NCS to APOA1 - for 89Zr-Al-HDL - or by incorporation of 1 % phospholipid-chelator DSPE-DFO at the expense of DMPC. Size and zeta-potential were measured on a Malvern NanoSeries Z-Sizer (Malvern, UK) in triplicate at 25 ºC, allowing equilibration for 120 s and with an angle of detection of 173º. Radiolabeling was achieved by reaction of the corresponding precursors with 89Zr-oxalate in PBS (pH 7.1-7.4) at 37 ºC for 2 h at an activity-to-APOA1 ratio of ~1 mCi/mg. The radiolabeled nanoparticles were purified by spin filtration and filtered through a 0.22 μm filter prior to use.

Radiochemistry. 89Zr was produced at Memorial Sloan-Kettering Cancer Center on an EBCO TR19/9 variable-beam energy cyclotron (Ebeo Industries Inc., British Columbia, Canada) via the 89Y(p,n)89Zr reaction and purified in accordance with previously reported methods to yield 89Zr with a specific activity of 5.3-13.4 mCi/μg.

HPLC and Radio-HPLC. HPLC was performed on a Shimadzu HPLC system equipped with two LC-10AT pumps and an SPD-M10AVP photodiode array detector. Radio-HPLC was performed using a Lablogic Scan-RAM Radio-TLC/ HPLC detector. Size exclusion chromatography was performed on a Superdex 10/300 column (GE Healthcare Life Sciences, Pittsburgh, PA) using PBS as eluent at a flow rate of 1 mL/min.

Preparation of modified HDL nanoparticles. Fluorescent HDL nanoparticles were prepared by incorporation of the NIRF dye DiR and modification with non-radioactive zirconium following a previously described method. Briefly, phospholipids (DMPC) and DiR were mixed in chloroform solution at a weight ratio of 97:3. A thin film was formed by evaporating the solvent, and large vesicles were made by hydrating the film with APOA1 solution. Subsequent sonication, centrifugation and filtration yielded a clear solution. These particles were modified with DFO-p-NCS and non-radioactive Zr to yield DiR@Zr-Al-HDL. Similarly, DiR@Zr-PL-HDL was prepared using the same procedure but adding 1 % DSPE-DFO to the expense of DMPC (instead of conjugating DFO-p-NCS), and subsequent reaction with non-radioactive zirconium. Both Zr-modified fluorescent HDL nanoparticles had the same retention time as their radioactive counterparts and as unmodified HDL (table S1).

Cy7-labeled HDL nanoparticles were prepared by reaction of rHDL with sulfo-Cy5.5 mono-NHS ester as follows. To a solution of rHDL in 0.1 M PBS (pH = 8.1) containing 7 mg/mL APOA1, the NHS ester was added to achieve a 6:1 molar ratio over APOA1. The mixture was stirred at room temperature for 90 min, after which time the labeled particles were purified by spin filtration using 10 kDa MWCO Vivaspin tubes and washed with PBS (5x2 mL). The number of Cy7 labels per APOA1 was ~4, as determined by spectrophotometry. The resulting fluorescent nanoparticles had the same retention time as unmodified rHDL on size exclusion chromatography.

Synthesis of Cy7-albumin. Cy7 mono-NHS ester (1.695 mL, 10 mg/mL in DMSO) was added to a solution of albumin from rabbit serum (16.425 mL, 10 mg/mL in PBS) under stirring at room temperature. After one hour, the reaction mixture was placed in a dialysis bag (3,000 MWCO) and dialyzed for 24 hours. Finally, the solution was washed from the remaining unbound dyes using centrifugal concentrators (10 kDa MWCO). The Cy7-albumin concentrate was diluted with PBS to a final concentration of 5 mg/mL.
Oxidation of HDL nanoparticles. In order to prepare oxidized HDL nanoparticles, the phospholipid DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) was included in the formulation at 4 mol % at the expense of DMPC. This modification had no effect on nanoparticle size as determined by size exclusion chromatography. Oxidized HDL (HDL-Ox) was prepared following a previously described method with slight modifications. Briefly, a PBS solution containing DOPC@HDL nanoparticles was stirred at 37 °C in the presence of EDTA, α-tocopherol and 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) for 20 h. The resulting oxidized product was washed thoroughly with PBS and subsequently modified by conjugation of DFO to apoA1 and radiolabeled with 89Zr as previously described to yield 89Zr-AI-HDL.

Flow cytometry. Female Apoe−/− mice (n = 4 per NP type and time point) fed a high fat diet for 18 weeks were injected with the corresponding fluorescent HDL analogs DiR@Zr-AI-HDL and DiR@Zr-PL-HDL at ~0.5 mg DiR/kg body weight. The nanoparticles were allowed to circulate for 24 or 48 hours and then aortas, spleen and blood were collected. Aortas and spleen were digested, and digested with a cocktail of enzymes, including liberase TH (Roche), hyaluronidase (Sigma-Aldrich), andDNase (Sigma-Aldrich), in a 37 °C oven for 24 h. A single-cell suspension was made by removing tissue aggregates, extracellular matrix, and cell debris from the solution. The same flow cytometry setting was applied to all samples. DiR was detected on the APC-Cy7 channel. To identify macrophages, monocytes, dendritic cells, neutrophils, and other immune cells, a lineage of antibodies recognizing CD90 (clone 53.2.1), B220 (clone RA3-6B2), CD49b (clone DX5), NK1.1 (clone PK136), Ly-6C (clone 1A8), Ter-119 (clone TER-119), and antibodies recognizing Ly-6C (clone AL21), F4/80 (BM8), and CD11c (N418) were used. All samples were measured on an LSRII (BD Biosciences, San Jose, CA) flow cytometer. Results were analyzed with FlowJo (Ashland, OR).

Pharmacokinetics and biodistribution in mice. Apoe−/− (18 weeks on HFD; n = 3 per NP type; weight: 26.7 ± 2.7 g) and wild type B6 (n = 3 per NP type; weight: 22.5 ± 0.9 g) mice received either 89Zr-AI-HDL or 89Zr-PL-HDL (0.19 ± 0.02 mCi, ~10 mg APOA1/kg) and the corresponding non-radioactive fluorescent analog at ~0.5 mg DiR/kg (~15 mg APOA1/kg) via the tail vein. At 2 and 30 min, and 2, 6, 24 and 48 h, blood (5-10 μL) was sampled from the tail vein. Radioactivity content was measured using a Wizard2 2480 automatic gamma counter (Perkin Elmer, Waltham, MA). For biodistribution studies, Apoe−/− (18 weeks on HFD; n ≥ 5 per NP type and time point; weight: 27.3 ± 3.5 g) and wild type B6 (n = 4 per NP type and time point; weight: 22.1 ± 1.8 g) mice were injected with either 89Zr-AI-HDL or 89Zr-PL-HDL (0.20 ± 0.03 mCi, ~10 mg APOA1/kg) and the corresponding non-radioactive fluorescent analog at ~0.5 mg DiR/kg (~15 mg APOA1/kg) via the tail vein. At 24 and 48 h, animals were sacrificed and perfused with PBS. Organs were excised, blotted and weighed before radioactivity counting on a Wizard2 2480 automatic gamma counter.

Micro-PET/CT Imaging. Mice (n = 28; 16 Apoe−/− mice, 18 weeks on HFD [weight: 27.8 ± 3.6 g] and 12 wild type B6 [weight: 22.1 ± 2.1 g]) were injected with either 89Zr-AI-HDL or 89Zr-PL-HDL at 8 mg APOA1/kg (0.20 ± 0.03 mCi, ~10 mg APOA1/kg) and the corresponding non-radioactive fluorescent analog at ~0.5 mg DiR/kg (~15 mg APOA1/kg) in 200 μL PBS solution via the tail vein. At 24h and 48h, animals were anesthetized with isoflurane (Baxter Healthcare, Deerfield, IL)/oxygen gas mixture (2%/ for induction, 1 % for maintenance), and a scan was then performed using an Inveon PET/CT scanner (Siemens Healthcare Global, Erlangen, Germany). Whole body PET static scans recording a minimum of 30 million coincident events were performed, with duration of 15 min. The energy and coincidence timing windows were 350-700 keV and 6 ns, respectively. The image data were normalized to correct for non-uniformity of response of the PET, dead-time compensation, positron branching ratio, and physical decay to the time of injection, but no attenuation, scatter, or partial-volume averaging correction was applied. The counting rates in the reconstructed images were converted to activity concentrations (percentage injected dose [%ID] per gram of tissue) by use of a system calibration factor derived from the imaging of a mouse-sized water-equivalent phantom containing 89Zr. Images were analyzed using ASIPro VMTM software (Concorde Microsystems, Knoxville, TN). Activity concentration correction of PET images was done using the built-in MR-based attenuation correction (MR-AC) map and images were reconstructed using the OP-OSEM algorithm. In addition, a time-of-flight (TOF) non-contrast enhanced angiography was performed for localization of arterial anatomical landmarks (renal arteries and iliac bifurcation). Imaging parameters were: TR, 1600 ms; TE, 118 ms; flip angle, 140 degrees; ETL, 83; slice thickness, 0.6 mm. For pharmacokinetic analysis, blood was sampled via the ear vein at 30 min, 24, 48, 72 and 120 h post injection. Blood was weighed and its radioactivity content measured on a Wizard2 2480 automatic gamma counter (Perkin Elmer, Waltham, MA). Radioactivity distribution measurements were performed at the end of the last scan (120 h p.i.) and 30 min after the injection of Cy7-albumin. Rabbits were sacrificed and perfused with saline. Organs were excised, blotted
and diced before weighing and counting using a Wizard² 2480 automatic gamma counter. Pigs (n = 3; weight: 61.2 - 63.5 kg) were injected with \( ^{89} \text{Zr}\)-PL-HDL (3.7 ± 0.3 mCi, n = 3) and rHDL (~40 mg total APOA1). At 48 h post injection, animals were anesthetized and underwent a whole-body PET/CT scan (PET acquisition time of 5 min/bed position; 9 beds) and a regional contrast-enhanced PET/CT scan (PET acquisition time of 15 min, 1 bed position). Using a power injector, 70 mL of iodine contrast agent was administered through an ear vein (flow rate 1.5 mL/sec) with a scan delay of 7 sec with the CT scan acquired in the cranio-caudal direction covering from the lower abdominal region to the knees.

Radioactivity distribution measurements were performed at the end of the imaging session. Pigs were sacrificed, organs were excised and harvested before weighing and counting using a Wizard² 2480 automatic gamma counter. Image analysis was conducted using OsiriX Imaging Software by drawing regions of interest (ROIs) on the selected tissues (liver, kidneys, spleen and abdominal aorta -from renal artery to iliac bifurcation-). Blood activity was quantified in the cardiac chambers. Standardized uptake values (SUVs) were obtained by averaging SUV\(_{\text{max}}\) values in each ROI drawn on all slices over the whole organ or over at least 10 slices of the tissue of interest.

Near-infrared fluorescence imaging. Shortly after sacrifice, perfused tissue samples were placed on thick black paper and imaged on an IVIS Spectrum Preclinical Imaging System (Perkin Elmer, Waltham, MA). Fluorescence images were acquired with excitation and emission wavelengths of 680 and 720 nm (Cy5.5), and 750 and 800 nm (Cy7 and DiR). Data were quantified as radiant efficiency.

 Autoradiography. Following sacrifice, animals were perfused and their aortas (mice and rabbits) or femoral arteries (pigs) excised and blotted. To determine radiotracer distribution, digital autoradiography was performed by placing tissue samples in a film cassette against a phosphorimaging plate (BASMS-2325, Fujifilm, Valhalla, NY) for 14 h (mouse aortas) or 48 h (rabbit aortas, pig femoral arteries) at -20 °C. Phosphorimaging plates were read at a pixel resolution of 25 μm with a Typhoon 7000IP plate reader (GE Healthcare, Pittsburgh, PA). Quantification was carried out using ImageJ software.

 Statistics. Data are presented as mean ± SD except for flow cytometry results, which are presented as mean ± SEM. Mann-Whitney tests were used to assess between group differences. To determine correlations, Spearman’s rho coefficients were calculated. A Bland-Altman plot was used to test for systematic bias between SUVs acquired by PET/CT and PET/MRI. The agreement between the successive PET/CT and PET/MRI scans was assessed using the intraclass correlation coefficient (ICC). For all tests, \( p < 0.05 \) represents statistical significance. Statistical analyses were performed with GraphPad Prism®, Version 6.0c (La Jolla, CA), and IBM SPSS Statistics 22.
References

Formation of methionine sulfoxide in apolipoproteins AI and AII is an early event that accompanies lipid peroxidation and can be enhanced by alpha-tocopherol. J. Biol. Chem. 1998;273:6080–7.

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Supplementary material and methods

Supplemental figures, tables and video can be found at http://imaging.onlinejacc.org/
Supplementary figures

Table S1. Composition (mol %), size (d.nm) and size exclusion chromatography retention time (min) for the different HDL nanoparticles used in this study.

<table>
<thead>
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<th></th>
<th>ApoA-I</th>
<th>DMPC</th>
<th>DSPE-DFO</th>
<th>DIR</th>
<th>Size</th>
<th>r.t.</th>
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<td>rHDL</td>
<td>1%</td>
<td>99%</td>
<td>-</td>
<td>-</td>
<td>8.5 ± 0.9</td>
<td>12.0 ± 0.2</td>
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<tr>
<td>89Zr-AI-HDL</td>
<td>1%</td>
<td>99%</td>
<td>-</td>
<td>-</td>
<td>8.6 ± 0.9</td>
<td>11.8 ± 0.5</td>
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<tr>
<td>89Zr-PL-HDL</td>
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<td>98%</td>
<td>1%</td>
<td>-</td>
<td>8.2 ± 0.9</td>
<td>12.0 ± 0.3</td>
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<tr>
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<td>97.5%</td>
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<td>-</td>
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<tr>
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<td>1%</td>
<td>96.5%</td>
<td>1.5%</td>
<td>-</td>
<td>-</td>
<td>12.0 ± 0.1</td>
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Figure S1. Gating procedure and cell targets of Zr-HDL nanoparticles in spleen and blood of Apoe⁻/⁻ mice (18 weeks on HFD), as determined by flow cytometry using non-radioactive fluorescent DiR-labeled analogs. Quantification data are shown in figure 1.

Figure S2. A) Whole-organ radioactivity in selected tissues for 89Zr-AI-HDL (top) and 89Zr-PL-HDL (bottom) at 24 h p.i. (n ≥ 4 per group). B) Representative 3D rendering PET/CT fusion images and radioactivity distribution in selected tissues for 89Zr-AI-HDL (top) and 89Zr-PL-HDL (bottom) at 48 h p.i. (n ≥ 4 per group). C) Whole-organ radioactivity in selected tissues for 89Zr-AI-HDL (top) and 89Zr-PL-HDL (bottom) at 48 h p.i. (n ≥ 4 per group). * P < 0.05; ** P < 0.01.

Figure S3. Standardized uptake values determined by PET/CT imaging at 1 h, 1, 2, 3 and 5 days p.i., and by gamma counting (γ) at 5 days p.i. in selected tissues for A) 89Zr-AI-HDL, and B) 89Zr-PL-HDL (n ≥ 3 per group). * P < 0.05.
Figure S4. Standardized uptake values determined by PET/MR imaging at 1 h, 1, 2, 3 and 5 days p.i., and by gamma counting ($\gamma$) at 5 days p.i., in selected tissues for A) $^{89}$Zr-AI-HDL, and B) $^{89}$Zr-PL-HDL (n ≥ 3 per group). * P < 0.05.

Figure S5. Correlation between standardized uptake values determined by A) PET/CT imaging, and B) PET/MR imaging, at 5 days p.i., and the ex vivo SUVs obtained by gamma counting after day 5 scan.

Figure S6. A) Athero-to-control SUV ratios for blood and aorta as determined by PET/CT for $^{89}$Zr-AI-HDL (top) and $^{89}$Zr-PL-HDL (bottom) (n ≥ 4 per group). B) Correlation between NIRF intensity (Cy5.5-HDL) and %ID/g in aortas at 5 days p.i. for $^{89}$Zr-AI-HDL (top, n = 9) and $^{89}$Zr-PL-HDL (bottom, n = 8). C) Correlation between NIRF intensity (Cy7-Albumin) and %ID/g in aortas at 5 days p.i. for $^{89}$Zr-AI-HDL (top, n = 9) and $^{89}$Zr-PL-HDL (bottom, n = 8).

Figure S7. A) Blood time-activity curve for $^{89}$Zr-AI-HDL in control rabbits (C) and for $^{89}$Zr-AI-HDL and $^{89}$Zr-AI-HDLOx in rabbits with atherosclerosis (A) (n = 4 per group). B) Radioactivity distribution in selected tissues for $^{89}$Zr-AI-HDL in control animals (C) and for $^{89}$Zr-AI-HDL and $^{89}$Zr-AI-HDLOx in animals with atherosclerosis (A) (n = 4 per group). C) Representative PET/CT images of $^{89}$Zr-AI-HDLOx at 1 h, 2 and 5 days p.i. in rabbits with atherosclerosis. D) Standardized uptake values determined by PET/CT imaging at 1 h, 1, 2, 3 and 5 days p.i., and by gamma counting ($\gamma$) at 5 days p.i., in selected organs for $^{89}$Zr-AI-HDL in control rabbits and for $^{89}$Zr-AI-HDL and $^{89}$Zr-AI-HDLOx in rabbits with atherosclerosis (n = 4 per group). E) Radioactivity concentration in aortas of control rabbits injected with $^{89}$Zr-AI-HDL -AI (C), and rabbits with atherosclerosis injected with $^{89}$Zr-AI-HDLOx -AIOx (A)- and $^{89}$Zr-AI-HDL -AI (A)- at 5 days p.i. determined by gamma counting (n = 4 per group). * P < 0.05; ** P < 0.01.