# The effect of loading nascent HDL with gadolinium phospholipids in the structural stability of the particles

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**Target audience**

Researchers in the fields of molecular imaging, contrast agents, lipoproteins, and atherosclerosis.

**Purpose**

We studied how increasing the amount of a gadolinium-labeled lipid during high density lipoprotein (HDL) nanoparticle synthesis affects its relaxivity and structural integrity. Our approach allows the optimization of the properties of Gd-labeled HDL as molecular imaging agent for atherosclerosis.

**Methods**

HDL nanoparticles were prepared using DMPC, MHPC (Avanti Polar Lipids), Gd-DTPA-DSA lipids as well as the apolipoprotein ApoA1 (donated by CSL Behring) as described elsewhere.[1] We varied the Gd-DTPA-DSA content from x=0 to x=0.33. Magnetic relaxivities where measured at 60 MHz (Bruker Minispec). Particle sizes were determined by DLS (ZETAPals, Brookhaven Instruments). Samples were also characterized by Transmission Electronic Microscopy (TEM).

**Results**

Gd-labeled HDL showed an increase in both r1 and r2 relaxivities with an increasing molar fraction ($x_{Gd}$) of Gd-DTPA-DSA (figure 1a), reaching a plateau at $x_{Gd}$=0.2 (r1 = 8.30 ± 0.31 $mM^{-1}s^{-1}$ ; r2 = 12.08 ±0 .69 $mM^{-1}s^{-1}$). The mean sizes of HDL also increased with $x_{Gd}$ from 8.4 ± 1.1 nm ($x_{Gd}$=0) to 44.9±20.2 nm ($x_{Gd}$=0.3) (figure 1b). Interestingly, when sizes of individual particles were plotted (figure 1c), two distinct populations were observed, A and B, at $x_{Gd}$ higher than 0.2. We found the mean sizes of these populations to be 8.2 ± 1.6 nm (n=143) and 51.7 ± 7.3 nm (n=86), respectively.

**Discussion**

The HDL MR molecular imaging agent is attractive for several reasons. It is composed of (mostly) natural components, has the ability to carry high payloads of lipophilic agents, including gadolinium-labeled lipids (e.g., Gd-DTPA-DSA), is relatively small and has natural stealth and targeting features. However, the structural characteristics of Gd-DTPA-DSA may affect the natural self-assembly of HDL-like particles, altering their morphological features. Our results indicate that when Gd-DTPA-DSA is used at molar ratio higher than 0.2, the ionic relaxivity of HDL nanoparticles does not increase. Furthermore, increasing this ratio caused larger aggregates, which are vesicular of nature and will likely display a different *in vivo* behavior. Figure 1b shows that particles of increasing sizes are formed at different molar fractions of Gd-lipids. A deeper analysis reveals that two different species are actually formed: HDL-sized particles with a mean size of ~8 nm and larger particles with a disk-like or vesicular structure. In-depth electron microscopy analyses are currently being performed.

**Conclusion**

Labeling HDL with Gd-DTPA-DSA must be cautiously performed. High payloads of this lipid do not necessary increase the relaxivity of HDL, while it favors the formation of larger nanoparticles, with a potential different *in vivo* behavior.

**References**

[1] Jonas A. *Methods Enzymol* 1986;**128**:553–82

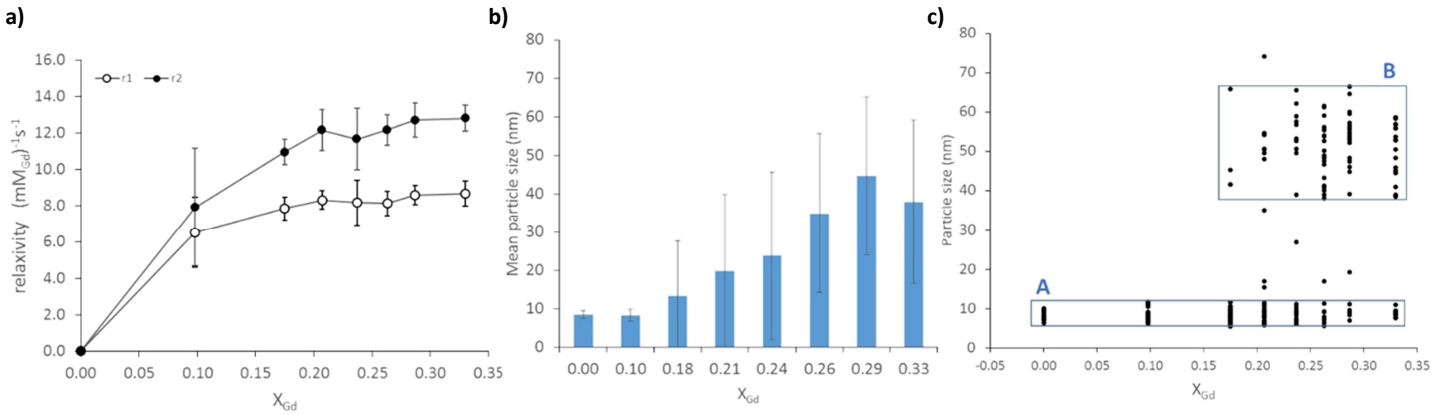


**Figure 1. a)** Variation of longitudinal (r1, hollow circles) and transversal (r2, solid circles) relaxivities (at 60 MHz) of HDL nanoparticles containing different molar fractions of Gd-labeled lipids. **b)** Mean size (±STDV) of 10 batches of HDL nanoparticles containing 8 different molar fractions of Gd-labeled lipids. **c)** Individual particle sizes showing the formation of 2 main populations of HDL particles: (A) with a size of 8.2±1.6 nm (n=143) and (B) with a size of 51.7±7.3 nm (n=86). The presence of two populations explains the large error bars observed in figure b.