

In vivo hyperpolarized lipophilic agents targeting atherosclerotic plaque

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Introduction MR hyperpolarization dramatically increases nuclear spin polarization to an order of unity, when nearly all nuclear spins contribute to the MR signal. This provides MR sensitivity enhancement by 10^4 - 10^6 fold compared to conventional MRI and MRS.

Purpose Here we utilize the dramatic sensitivity gain of Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment (PASADENA) to study the utility of ^{13}C hyperpolarized lipophilic reagents in low density lipoprotein receptor deficient (LDLR) mice, which develop massive accumulation of lipids in aortas when on a high fat diet.

Methods We utilized PASADENA to obtain hyperpolarized 2,2,3,3-tetrafluoropropyl 1- ^{13}C -propionate (TFPP) using the double bonded molecular precursor 2,2,3,3-tetrafluoropropyl 1- ^{13}C -acrylate (TFPA) [3]. PASADENA hyperpolarization of TFPP yields aqueous solutions with 15-20% ^{13}C polarization, which is similar to our rate for hyperpolarized 2-hydroxyethyl propionate [1] and succinate [2]. Due to TFPP's low solubility in water, the reagent was polarized in ~30-37% EtOH solution using a procedure previously described [1,2]. In this study, we injected 1 mL of 10-25 mM TFPP ^{13}C hyperpolarized solutions into the heart of LDLR deficient mice. In addition, we conducted *ex vivo* ^{19}F binding assays of LDLR and control mouse aortas using the same injected solution and straight TFPA solutions by methods developed earlier [3].

Results The *in vivo* ^{13}C spin lattice relaxation time of hyperpolarized TFPP T_1^* varies between 14 seconds and 22 seconds. The ^{13}C spectrum (Fig. 1), recorded shortly after injection of hyperpolarized solution yielded two resonances. The resonance at ~176 ppm corresponds to solution TFPP, whereas the resonance at ~172.5 ppm is the spectroscopic signature of TFPP in slow exchange with lipids in the aorta. This conclusion is based on our previous observations of the ^{19}F resonances in synthetic lipid membranes [3]. These membranes, when mixed *in vitro* with ^{13}C hyperpolarized TFPP, exhibited a similar spectroscopic pattern (not shown here). Additionally, we found a consistent difference in the ratio of 172.5 ppm and 176 ppm resonance integral intensities between LDLR deficient mice (5.3%) and controls (1.9%). This ratio can potentially serve as an indicator of lipid deposits in aortas. Additional experiments are in progress in our laboratory to provide statistical evidence for this hypothesis.

After *in vivo* experiments, the aorta of the animals were harvested and stored in saline solution to remove blood from the vessels. The same solution of previously hyperpolarized TFPP was added to aortic sample in a 4 mm Magic Angle Spinning (MAS) rotor. ^{19}F fast (>6 kHz) MAS spectra were recorded (Fig. 2). The spectra demonstrate (i) TFPP resonance in solution and lipid interactions and, more importantly, (ii) atheroma (LDLR) specimens exhibited a high lipid/solution resonance ratio, similar to our *in vivo* results described above. These *ex vivo* results prove that hyperpolarized TFPP is indicative of lipid accumulation in arterial wall rather than any other lipophilic compounds of blood. Moreover, when the pure TFPA agent is used, eliminating the use of EtOH, the ^{19}F lipid resonance dominates the spectrum (Fig. 2). This observation suggests that pure hyperpolarized TFPP agent (work in progress in our laboratory) would provide much greater MR sensitivity to detect plaque deposits *in vivo*.

Discussion We demonstrated the feasibility of applying lipophilic hyperpolarized ^{13}C agents *in vivo*. We conclude that hyperpolarized ^{13}C TFPP spectroscopy can detect and potentially quantify atherosclerotic plaque *in vivo*.

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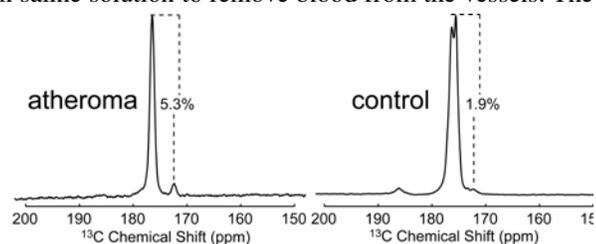


Figure 1. ^{13}C PASADENA in vivo enhanced NMR spectra demonstrating binding/intermediate exchange of molecular agent TFPP in LDLR deficient mice (injection in heart).

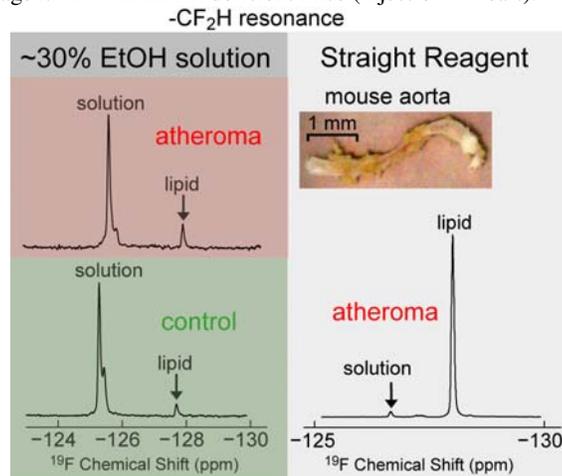


Figure 2. ^{19}F *ex vivo* NMR spectra demonstrating binding/intermediate exchange to LDLR deficient mouse aortas. Spectra recorded using Magic Angle Spinning (MAS) > 6 kHz without proton decoupling at 11.7T.