

## USPIO enhanced MRI in NAFLD; a feasibility and proof of concept study

Loek P. Smits<sup>1</sup>, Bram Coolen<sup>2</sup>, Wouter Nijhof<sup>1</sup>, Jurgen H. Runge<sup>2</sup>, Erik S. Stroes<sup>1</sup>, and Aart J. Nederveen<sup>2</sup>

<sup>1</sup>Department of Vascular Medicine, AMC, Amsterdam, Netherlands, <sup>2</sup>Department of Radiology, AMC, Amsterdam, Netherlands

**Introduction:** The differentiation between simple steatosis and steatohepatitis (NASH) in subjects with NAFLD is clinically important, since simple steatosis is a relatively benign disorder, whereas NASH may result in fibrosis, cirrhosis and hepatocellular carcinoma. Currently, diagnostic techniques can identify subjects with steatosis, fibrosis and cirrhosis, but not NASH. Superparamagnetic particles of iron-oxide (SPIOs) have been used as a MRI contrast agent in NAFLD, and showed great promise to differentiate between NASH and simple steatosis<sup>1-3</sup>. Subjects with NASH had a decreased hepatic uptake of SPIOs, presumably mediated by a decreased phagocytic capacity of the Kupfer cells<sup>4</sup>. Ultrasmall SPIOs (USPIOs) are a different class of iron-oxide particles, with some distinct pharmacokinetic properties compared to SPIOs, most important a reduced hepatic uptake and a longer circulating plasma half-life. Since SPIOs are not available for clinical use anymore, and ferumoxytol (an USPIO) has recently been approved, the aim of this study was to find the optimal dosing and timing for USPIO-MRI in NAFLD, and to investigate whether patients with NASH have a reduced hepatic uptake of USPIOs.

**Methods:** Quantitative T2\* MRI scans (single slice, breathhold, multi-echo gradient echo, fat suppressed, voxel size 2x2mm, TE 1.6 ms,  $\Delta$ TE 4 ms, 10 echoes, TR 120 ms, slice thickness 5 mm) were performed at baseline and at several timepoints following different dosages of ferumoxytol (1.8 and 3.6 mg/kg lean body mass (lbm)) in six healthy volunteers to find optimal dosing and timing of USPIO-MRI in NAFLD. Scans were acquired from the liver to assess hepatic uptake of USPIO and from the renal cortex and blood pool to assess the presence of ferumoxytol in the blood. MRI scans were performed on a 3T Philips Ingenia scanner. For image analysis, segmentation of the liver, renal cortex and cardiac blood pool was performed manually using in house developed software in MATLAB. For the liver, large vessels were masked out using signal thresholding. In addition to T2\* imaging, quantitative T2 measurements using a multi-spin echo sequence (single slice, breathhold, fat suppressed, voxel size 4x4mm, TE 5.5 ms,  $\Delta$ TE 5.5 ms, 10 echoes, TR 350 ms, slice thickness 5 mm) were performed in the liver only. T2<sup>(\*)</sup> was calculated by fitting the multi-echo signal intensities to a mono-exponential decay function. The amount of USPIO in a specific ROI was quantified by the difference in R2<sup>(\*)</sup> (1/T2<sup>(\*)</sup>) between the post-contrast scan and the baseline scan ( $\Delta$ R2<sup>(\*)</sup> = R2<sup>(\*)</sup>post - R2<sup>(\*)</sup>baseline). To demonstrate a reduced hepatic USPIO uptake in patients with NASH, quantitative T2\* (and T2 for liver only) MRI scans of the liver and renal cortex were performed in five subjects with biopsy-proven NASH and five age matched controls at baseline, t=5 minutes and t=72 hours. Differences in  $\Delta$ R2<sup>(\*)</sup> between groups were statistically tested using the students t-test for independent samples in SPSS.

Figure 1. Representative images (1A) and fits (1B) of liver T2\* at baseline and 72h post-contrast in a healthy volunteer

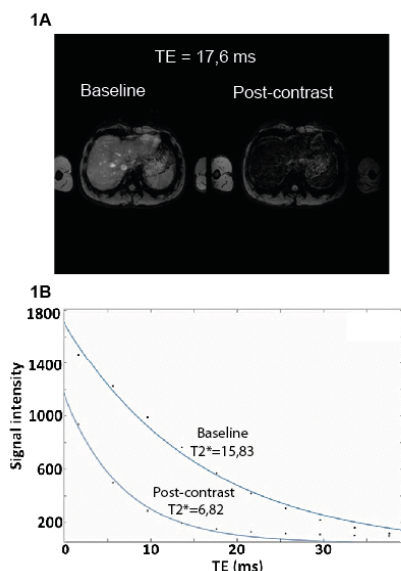


Figure 2. Dosis and timing optimisation of USPIO-MRI, showing the  $\Delta$ R2\* in the liver after different dosages of ferumoxytol over time (2A) and the  $\Delta$ R2\* of the liver and blood pool after 3.6 mg/kg LBM ferumoxytol over time

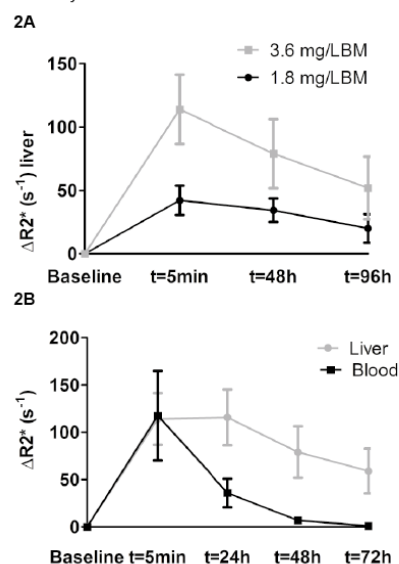
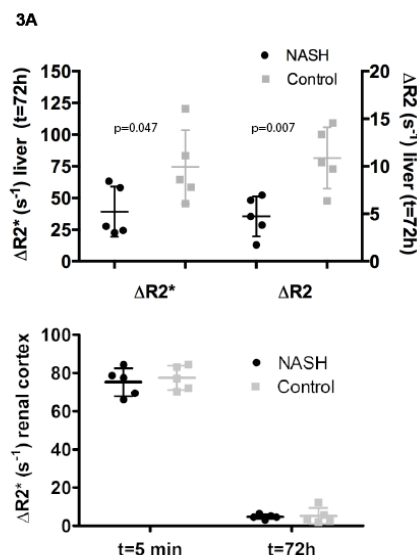


Figure 3. Patient study with USPIO-MRI in NAFLD, showing the  $\Delta$ R2\* in the liver (3A) and renal cortex (3B) after administration of 3.6 mg/kg LBM ferumoxytol in NASH patients compared to controls.



**Results:** Ferumoxytol administration resulted in a larger increase in R2\* of the liver in the high-dose group compared to the low-dose group ( $51.9 \pm 24.8$  s<sup>-1</sup> vs.  $20.1 \pm 11.2$  s<sup>-1</sup> at t=96h, figure 2A) in healthy volunteers. Since we want to demonstrate a decreased hepatic uptake of USPIO in NASH, and the R2\* increase in the low-dose group is relatively mild in control subjects, we do not expect to find this difference using a low-dose. Hence we choose to continue the study with the high dose. To evaluate at which time-point the R2\* increase in the liver following USPIO administration is effectuated solely by hepatic uptake, and not hepatic perfusion of blood rich in USPIOs, we measured the R2\* increase in the liver and blood pool at several time-points after contrast administration. As illustrated in figure 2B, the blood pool R2\* almost returned to baseline 72 hours after the administration of ferumoxytol, where the liver R2\* liver remains elevated compared to baseline. The R2\* increase in the liver at t=72h thus reflects hepatic USPIO uptake, since nearly all USPIOs are cleared from the blood. Therefore, to evaluate differences in hepatic USPIO uptake between NASH subjects and controls, we choose to perform MRI-scans of the liver at baseline and 72 hours after USPIO administration. Since increase in blood pool R2\* correlated well with the increase in renal cortex R2\* (well perfused organ without USPIO uptake, data not shown), we choose to use renal cortex  $\Delta$ R2\* as a surrogate for circulating USPIO concentration. Five subjects with NASH (mean age 57.2 yr) and five age matched controls (mean age 56.5 yr) were included in the patient study. Subjects with NASH had a significantly lower increase in liver R2\* ( $39.2 \pm 19.8$  s<sup>-1</sup> vs.  $74.5 \pm 29.1$  s<sup>-1</sup>, p=0.047) and, even more consistent, in liver R2 ( $\Delta$ R2 of  $4.7 \pm 2.1$  s<sup>-1</sup> vs.  $10.9 \pm 3.2$  s<sup>-1</sup>, P=0.007) 72 hours after the administration of ferumoxytol (figure 3A). There was no difference in R2\* increase in the renal cortex at t=5 min, indicating a comparable plasma concentration of ferumoxytol between groups. At t=72h, the  $\Delta$ R2\* almost returned to zero in both groups, indicating nearly no circulating ferumoxytol in both groups anymore (figure 3B).

**Conclusion / discussion:** In the present study, we showed the feasibility of USPIO-MRI in NAFLD, and demonstrated a reduced hepatic USPIO uptake in patients with NASH compared to healthy controls. Previous studies found SPIO enhanced MRI capable of differentiating NASH from simple steatosis<sup>1-3</sup>, based on a reduced hepatic SPIO uptake in NASH. Since we showed a decreased hepatic USPIO uptake in NASH, this may implicate that USPIO-MRI is also able to identify subjects with NASH. Further research comparing patients with simple steatosis and NASH with the current USPIO-MRI protocol is necessary to fulfill this promise.

**References:** 1. Asanuma, Gut (2010); 2. Tomita, JMIR (2008); 3. Tonan, JGH (2012); 4. Miyata, WJG (2011)