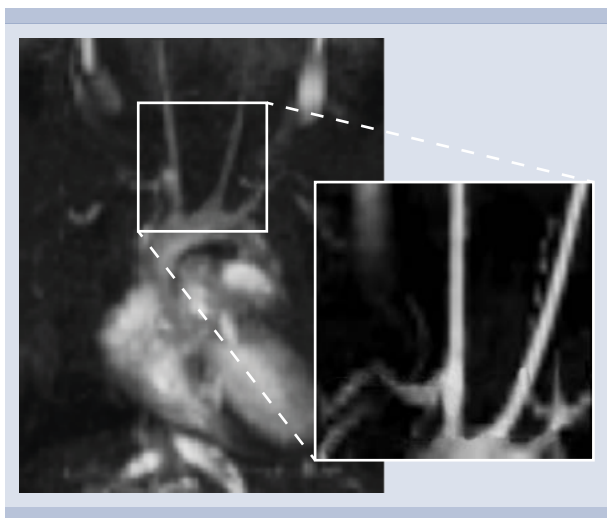


MRI of atherosclerotic plaque



In vivo longitudinal monitoring of plaque progression by GadoSpin™ F

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Introduction

Atherosclerosis is the condition in which arteries narrow and harden due to an excessive build-up of inflammatory plaque around the inner arterial wall. The disease disrupts the flow of blood around the body, thereby posing a serious risk for cardiovascular disease including myocardial infarction, heart failure and stroke¹. Over the past two decades our understanding of the pathological mechanism

of atherosclerosis has rapidly advanced and magnetic resonance imaging (MRI) has emerged as a leading non-invasive imaging modality of atherosclerotic disease².

In this study we develop an MRI method using the innovative MRI agent GadoSpin™ F to quantify plaque burden in apolipoprotein E-deficient (ApoE^{-/-}) mice. Through measurements of the plaque size and contrast-to-noise ratio (CNR) at various disease stages, we assess the feasibility of the method to non-invasively detect atherosclerotic plaque and longitudinally monitor its progression.

Materials and methods

Experiments were performed on isoflurane-anaesthetized ApoE^{-/-} mice (n = 5, 16 weeks old) and wild type mice (control, n = 2, 16 weeks old) fed on a high-fat diet (HFD, ssniiff Spezialdiäten GmbH, Soest, Germany) for a period of 20 weeks. To follow disease progression, imaging was performed on the animals at 5, 10, 15, and 20 weeks after starting the HFD using a 7 T preclinical MRI scanner (Bruker ClinScan, Ettlingen, Germany). A 4-channel mouse surface coil (20 mm diameter) was used as receiver, and a rat body coil was used as transmitter (Bruker, Ettlingen, Germany).

For monitoring plaque progression, MR images were obtained before and 2 h after tail vein injection (100 µmol Gd/kg body weight) of GadoSpin™ F (Viscover™, nanoPET Pharma GmbH, Berlin, Germany), a Gd-containing amphiphilic MRI agent that accumulates in atherosclerotic plaques enabling T₁-weighted visualization of plaque burden. A 2D T₁-weighted turboflash sequence (T_R = 2450 ms, T_E = 2.6 ms T₁ = 1450 ms, FA = 20°, effective voxel resolution 0.09 x 0.09 x 0.09 mm³, 1 slice) covering the aortic arch was performed to obtain parasagittal images. Using the parasagittal scan, transverse planes were selected and a 3D T₁-weighted inversion recovery turboflash sequence (T_R = 650 ms, T_E = 2 ms T₁ = 250 ms, FA = 20°, effective voxel resolution 0.18 x 0.18 x 0.18 mm³, 64 slices) was performed. To estimate plaque size and CNR, regions of interest (ROI) in each transverse slice were placed in the muscle and in the atherosclerotic plaques of the aortic root and brachiocephalic artery. The plaque size was determined by addition of the

plaque ROI of each slice. The CNR was calculated using the signal intensities in the plaque and muscle, according to the following equation:

$$CNR = \frac{SI_{\text{plaque}} - SI_{\text{muscle}}}{0.5 \times (\sigma_{\text{plaque}} + \sigma_{\text{muscle}})}$$

To monitor blood clearance of GadoSpin F, ApoE^{-/-} mice (n = 3, 16 weeks old) were injected with the imaging agent (100 µmol Gd/kg body weight) and scanned every 10 min for a period of 130 min using the 3D inversion recovery turboflash sequence described above. ROI were placed in the ascending aorta and muscle, and the signal intensities were determined at the varying timepoints and normalized to the signal intensities before application of the imaging agent. Throughout, MR image analysis was conducted using ImageJ software (National Institutes of Health, Maryland, USA). Statistical analysis (details reported elsewhere³) was performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA).

Results and discussion

In order to determine the optimal time delay between GadoSpin F injection and imaging of plaque in ApoE^{-/-} mice, the contrast-induced signal enhancement in blood was measured over a period of 130 min. The signal intensity within the blood showed a maximum at 10 min post injection of the imaging agent and was found to decrease continuously over time (Fig. 1). Since the imaging agent was found to be completely cleared from the blood at 2 h post injection, this imaging timepoint was selected for MR imaging of plaque. So as to monitor disease progression by MRI, animals on an HFD for 5, 10, 15, and 20 weeks were imaged before and 2 h after injection of GadoSpin F. Whereas no signal enhancement was detected in wild type mice, significant

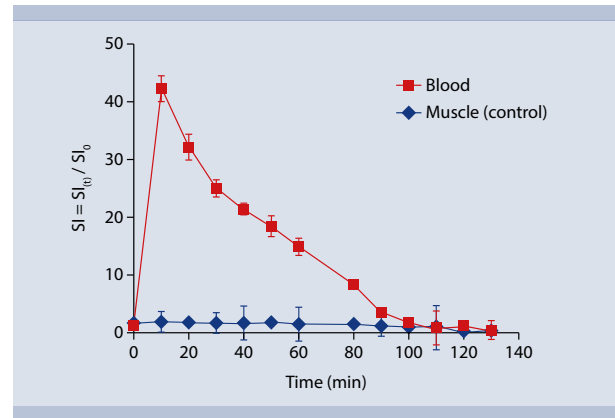


Figure 1: Blood clearance of GadoSpin F by MRI.

Time course of contrast enhancement in blood and muscle after intravenous injection of GadoSpin F.

signal enhancement was observed in the atherosclerotic plaques of the aortic root and brachiocephalic artery of ApoE^{-/-} mice (Fig. 2 and 3). Plots of the time-dependent CNR at both examined locations showed that, for the ApoE^{-/-} mice, the CNR progressively and significantly increased with increasing duration of the HFD. In particular, the temporal changes in plaque CNR of the aortic root were significantly different between mice on an HFD for 10 and 15 weeks ($p < 0.001$) and for 15 and 20 weeks ($p < 0.001$), whereas no significant CNR differences were detected between mice on an HFD for 5 and 10 weeks (Fig. 2). The CNR for brachiocephalic plaque at various timepoints during the HFD regimen showed significant differences between the mice on an HFD for 5 and 10 weeks ($p < 0.01$) and 15 and 20 weeks ($p < 0.001$), whereas no significant difference was measurable between mice fed on an HFD for 10 and 15 weeks (Fig. 3). Thus, significant differences in the plaque CNR of both the aortic root and the brachiocephalic artery were detected at almost all investigated timepoints, despite the short time interval of 5 weeks.

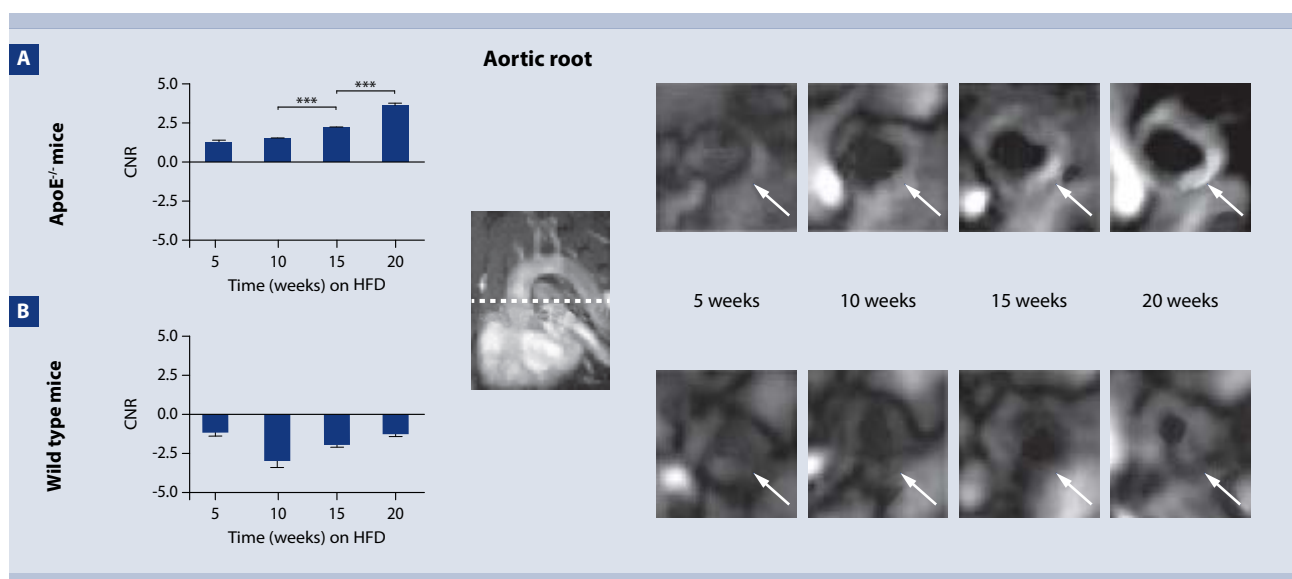


Figure 2: Monitoring of plaque progression in the aortic root via contrast-to-noise ratio (CNR) estimation.

A. The temporal changes in plaque CNR of the aortic root 2 h after injection of GadoSpin F show that in ApoE^{-/-} mice the CNR progressively increases with increasing duration of the HFD. Significant differences in the CNR are observed between 10 and 15 weeks ($p < 0.001$) and 15 and 20 weeks ($p < 0.001$), whereas no significant differences are detectable between 5 and 10 weeks. **B.** No signal enhancement is detected in the aortic root of wild type mice. The white arrows on the MR images point to the vessel wall of the aortic root.

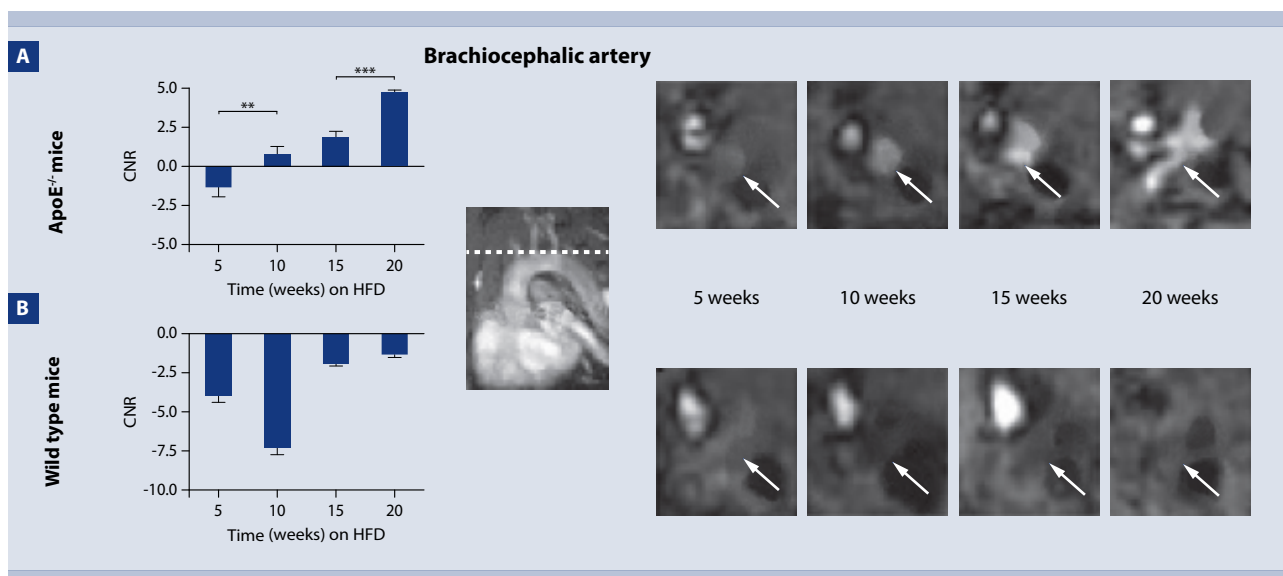


Figure 3: Monitoring of plaque progression in the brachiocephalic artery via contrast-to-noise ratio (CNR) estimation.

A. The CNR of the brachiocephalic plaque 2 h after injection of GadoSpin F in ApoE^{-/-} mice increases with increasing duration of the HFD. Significant differences are observed between 5 and 10 weeks ($p < 0.01$) and 15 and 20 weeks ($p < 0.001$), whereas the difference between 10 and 15 weeks is not significant. **B.** No signal enhancement is detected in the brachiocephalic artery of wild type mice. The white arrows on the MR images point to the vessel wall of the brachiocephalic artery.

These temporal changes in plaque CNR were found to be in agreement with the estimated progression in plaque size (Fig. 4). In the aortic root, plaque size increased significantly from $0.52 (\pm 0.01) \text{ mm}^3$ at 5 weeks on an HFD to $2.25 (\pm 0.01) \text{ mm}^3$ at 20 weeks on an HFD. In the brachiocephalic artery the time-dependent plaque size also progressively increased with time spent on the HFD, from $0.15 (\pm 0.01) \text{ mm}^3$ at 5 weeks to $0.54 (\pm 0.02) \text{ mm}^3$ at 20 weeks, yet the increase was not as significant as in the aortic root. Interestingly, the correlation between CNR and plaque size was found to vary depending on the plaque location: In the brachiocephalic artery this correlation was found to be moderate ($r > 0.68$; $p < 0.01$; 95% confidence interval), whereas that in the aortic root was found to be relatively strong ($r > 0.78$; $p < 0.001$, 95% confidence interval). These findings imply that, with the aid of GadoSpin F, plaque development at various stages of atherosclerotic disease can be assessed by measurement of the CNR which, in turn, is correlated to the plaque size.

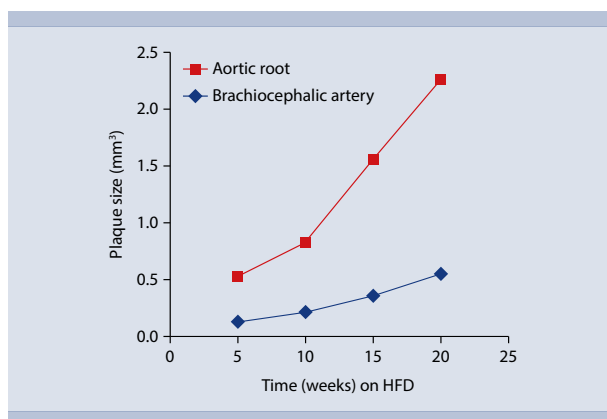


Figure 4: Monitoring of plaque progression via plaque size estimation. The plaque size in the aortic root significantly increases with time spent on the HFD. In the brachiocephalic artery, the plaque size also increases over time, yet not as significantly as in the aortic root.

Conclusion

In this study we developed an MRI method using the innovative MRI agent GadoSpin F to monitor plaque progression in ApoE^{-/-} mice. Through measurements of the plaque size and contrast-to-noise ratio (CNR) at various disease stages, we demonstrated that MRI monitoring of plaque burden within the same animal is feasible even at short time intervals of 5 weeks. This method, therefore, represents a reliable tool for preclinical longitudinal MRI of atherosclerosis and may assist in the discovery of novel therapeutic interventions for atherosclerotic disease.

Viscover™ Product	Order No.
GadoSpin™ M, 1 x 5 injections	130-095-134
GadoSpin™ M, 5 x 5 injections	130-095-135
GadoSpin™ P, 1 x 5 injections	130-095-136
GadoSpin™ P, 5 x 5 injections	130-095-137
GadoSpin™ F, 1 x 5 injections	130-095-162
GadoSpin™ F, 5 x 5 injections	130-095-163
GadoSpin™ D, 1 x 5 injections	130-095-164
GadoSpin™ D, 5 x 5 injections	130-095-165

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