High-Resolution Multi-Contrast MRI Characterization of Rabbit Atherosclerosis using Clinical Pulse Sequences

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Introduction: Atherosclerosis is the leading cause of morbidity and mortality in developed countries through its association with stroke, myocardial infarction, and sudden cardiac death. Atherosclerosis is regarded as an inflammatory disease where the vessel wall thickens and develops a lipid necrotic core with overlaying fibrous tissue[1]. Animal models to study the natural disease progression seen in humans have been lacking as most models involve some form of invasive acute injury or genetic manipulation. The long-term low-cholesterol-enriched rabbit model has been studied at the histological level of characterization and shown to develop human-like lesions with 24 weeks of feeding[2]. However, histology combined with MRI characterization of this model is lacking, especially with respect to regional plaque development and there has been little research following this model out to very long term (>18 months on diet)[3]. Multi-contrast high resolution MRI performed at 1.5T has been found to be an accurate method for assessing plaque structure and composition in carotid endarterectomy specimens[4]. The present study set out to characterize the long-term low-cholesterol-enriched rabbit model of atherosclerosis using high resolution multi-contrast *ex vivo* MRI and to determine regional variation of plaque complexity throughout the aorta. Eventually this research could be used as a tool for visualizing the vulnerable plaque and direct future *in vivo* research by specifying target regions in the aorta for imaging.

Methods: New Zealand White rabbits were fed 100 g/day of an atherogenic diet supplemented with 0.125%-0.25% cholesterol titrated to maintain a serum cholesterol concentration between 600 and 1000 mg/dl for 27 months. Control rabbits were fed 100 g/day of normal chow. Rabbits were euthanized using a lethal dose of a ketamine xylazine mixture and aortae were collected according to region. The ascending aortic arch, descending aortic arch, mid-thoracic aorta, and abdominal aorta were fixed for 24 hours in 10% formalin and stored in PBS. High resolution *ex vivo* MRI was performed using a 3.0 T Signa Excite HD GE MR scanner interfaced with a custom insert gradient coil and a solenoid RF coil. Multi-contrast images were collected using a fast-spin-echo (FSE) sequence (resolution=104µm x 104µm x 400µm, NEX=2) weighted for T1 (TE=10 ms, TR=800 ms, BW=±16 kHz, ETL=4, Scan Time~4:00), T2 (TE=50 ms, TR=3000 ms, BW=±16 kHz, ETL=14, Scan Time~5:00), and proton density (TE=10 ms, TR=3000 ms, BW=±16 kHz, ETL=6, Scan Time~4:00); a fast imaging employing steady-state acquisition (FIESTA) sequence (TE=4.2 ms, TR=8.5 ms, resolution=100µm x 100µm x 200µm, BW=±16 kHz, Flip Angle=20, NEX=2, Scan Time~3:00); and a diffusion weighed echo planar imaging (DW-EPI) sequence (TE=50 ms, TR=4000 ms, resolution=200µm x 200µm x 800µm BW=±16 kHz, b-value=1000, NEX=2, Scan Time=2:08). To validate the structures detailed with MRI, correlative paraffin embedded sections stained with H&E and Alizarin red were prepared.

Results: Multi-contrast high resolution MRI of this long-term low-cholesterol-enriched rabbit model reveals complex human-like lesions with a prominent multilayered appearance in all regions of the aorta that we studied. Qualitatively the most complex and thickest lesions developed in the descending aortic arch (Figure 1A). Focal regions of signal loss are characteristic of calcification deposits or cholesterol crystal accumulation and are not easily detected with most *in vivo* MRI; however this was shown in the descending and mid-thoracic aorta on the ex vivo T2W FSE images. Hematoxylin and eosin staining of correlative sections showed complex structure with a fibrous cap and necrotic core. This is particularly evident in the descending aortic arch. Alizarin red staining for calcium showed calcium deposits in most regions of the aortic plaque adjacent to the media layer (not shown) and this corresponded to the darkest regions seen in the MR images. Age-matched control rabbits showed no regions of intimal thickening and very little structural definition in the vessel wall as shown with T2W imaging (Figure 1B).

Discussion: In this study we performed multi-contrast *ex vivo* MR images of rabbit atherosclerosis at multiple locations of the aorta and made qualitative observations of intraplaque composition. Furthermore, our results show strong regional variation in plaque complexity, which suggests that the upper descending aorta is the most complex and thickest, making it a prime target region for future *in vivo* MR imaging studies, especially those focused on plaque compositional analysis. Typically, both *in vivo* and *ex vivo* MRI studies have focused on the abdominal aorta where lesions are less complex[3,5]. Attempts to image the upper aorta have been hampered by the increased motion (cardiac, respiratory) present in this region. Successful in vivo imaging in this region will require further improvements in image quality through advances in cardiac and respiratory gating. This work also has implications for helping define the most appropriate standard and novel sequences for plaque characterization in rabbit and, by extension, human atherosclerosis.

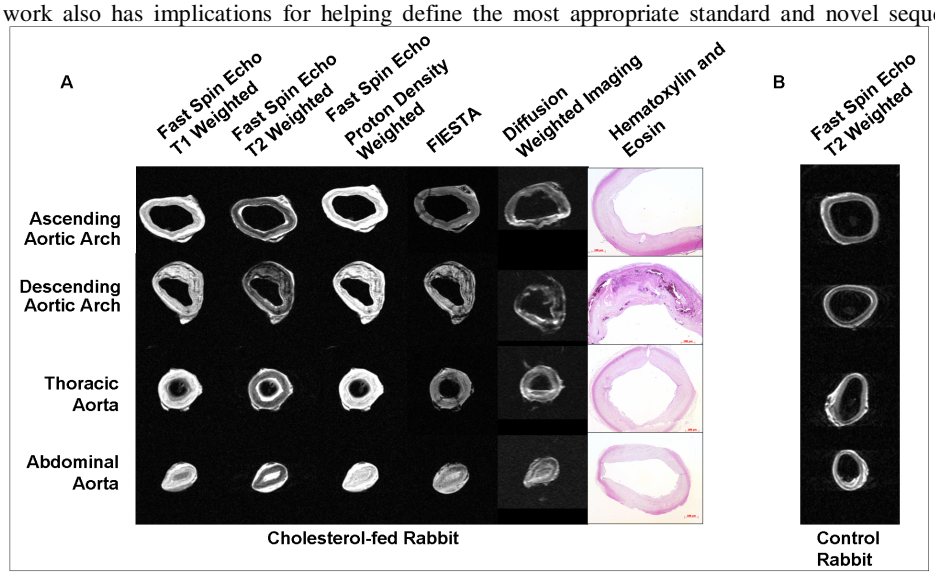

Figure 1. Multi-contrast MRI of aortic atherosclerosis. A. Profound intimal thickening in all regions of the aorta were detected with the most prominent thickening being seen in the descending aortic arch (2nd row). Lesions appear to be most complex in the descending aortic arch with distinct multi-layered appearance and regions of signal loss. Histology from the same region defines the multi-layered appearance as a fibrous cap with a necrotic core. **B.** T2W FSE of a control chow fed rabbit shows no intimal thickening in all regions of the aorta. The bright surrounding region corresponds to the adventitia and the darker interior region of the vessel wall is the media.

References: **1**. Ross R. (1993) Nature. 362: 801-809. **2**. Daley SJ et al. (1994) Arterioscler Thromb. 14: 95-104. **3**. Ronald JA et al. (2007) J Magn Reson Imaging. 26(4): 1010-9. **4**. Clarke SE et al. (2003) Magn Reson Med. 50: 1199-1208. **5**. Helft G et al. (2002) Circulation. 105(8): 993-8.