Quantitation of Atherosclerosis *in vivo* in LDLR Knockout Mice by 3D MRI

P.D. Hockings, T.J. Roberts, D.G. Reid, M. Vidgeon-Hart, D.A. Harris, P.H.E. Groot, K.E. Suckling, G.M. Benson
GlaxoSmithKline, The Frythe, Welwyn, Herts, AL6 9AR, UK

Abstract A 3D fast spin echo MRI method was developed to measure atherosclerotic plaque and lumen area in the innominate artery of low density lipoprotein receptor deficient (LDLR(-/-)) and control mice. Mean MRI plaque area was 0.14±0.09 and 0.02±0.04 mm^2 for LDLR(-/-) and control, respectively, compared to 0.31±0.08 and 0.07±0.01 by histology. Correlation between the two sets of measurements gave a R^2 of 0.64. The similarities in lumen area combined with the differences in plaque area indicate a degree of arterial remodelling.

Introduction Investigation of the size and morphology of atherosclerotic plaques *in vivo* is important both in humans and in animal models. In particular, plaque development in the thoracic aorta and innominate artery of patients and mice is a common cause of embolic events[1,2]. Previous *in vivo* studies of plaque in the mouse have concentrated on the abdominal aorta where plaque development is slower but movement associated with the respiratory and cardiac cycles is not so severe[3]. We have developed an *in vivo* 3D fast spin echo magnetic resonance imaging method that allowed us to measure atherosclerotic plaque in the innominate artery and aortic arch in low density lipoprotein receptor deficient mice (LDLR(-/-)).

Method All experiments complied with the Animals (Scientific Procedures) Act, 1986, UK. Nine transgenic LDLR(-/-) mice and 9 control C57Bl mice were imaged 12 weeks after the LDLR(-/-) mice were transferred to a high fat diet. Images were acquired on a Bruker AMX300 interfaced to an 18.3 cm 7T magnet as previously described[4]. Briefly, segmented 3D Fast Spin Echo image acquisition was synchronised with the respiratory and cardiac cycles of free breathing mice anaesthetised with isoflurane. 3D images were acquired during the systolic phase of the cardiac cycle with (FS) and without fat suppression (NFS). TE(eff)/TR were 13/800 ms and isotropic resolution 140 μm. The isotropic resolution of the 3D data sets was increased to 70 μm by interpolation and they were re-orientated so that slices through the data sets were orthogonal to the innominate artery. AnalyzeAVW software (Biomedical Imaging Resource) was used to combine the NFS and FS images into the red and green channels of a 3D RGB image and segment the innominate artery into lumen and plaque over its entire length i.e. between the aortic arch and the right sub-clavian artery. Data was analysed in a blinded fashion.

After removal from the magnet the mice were killed, perfuse fixed and sections taken for histology. Histological sections (5 μm) were taken every 50 μm along the innominate artery and stained with haematoxylin and eosin. Arterial lumen and wall ("plaque") areas were quantified using Optimas image analysis software run on a PC.

Results Both NFS and FS images acquired in young LDLR(-/-) mice fed a high fat diet showed the development of atherosclerotic plaque in the innominate artery (Figure 1). The length of the innominate artery varied markedly between mice so results are reported as mean areas of plaque and lumen and summarised in Table 1. Plaque areas measured by MRI were systematically lower than those measured by histology. This may be partially attributable to the difficulty of segmenting plaque adjacent to regions of isointensity such as the thymus. Lumen areas are much higher by MRI as would be expected from images acquired in systole compared to the flaccid post-mortem condition. Correlation between plaque measurements gave a R^2 of 0.64 (Figure 2).

Conclusion There is significant correlation between MRI and histological plaque measurements and both techniques report significant differences in innominate artery plaque between LDLR(-/-) and control mice. The similarities in lumen area combined with the differences in plaque area indicate a degree of arterial remodelling.

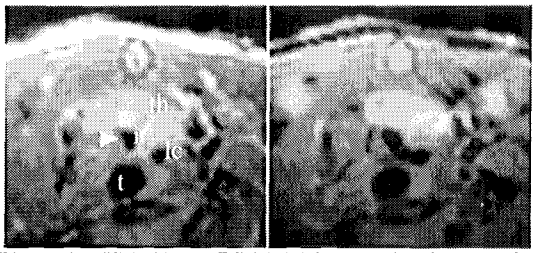

Figure 1 NFS (left) and FS (right) images showing an axial slice orthogonal to the innominate artery of a LDLR(-/-) mouse at week 12. The plaque (arrowhead), innominate artery (i), left carotid (lc), thymus (th) and trachea (t) are indicated.

	MRI		Histology	
	plaque (mm^2)	lumen (mm^2)	wall area (mm^2)	lumen (mm^2)
LDLR(-/-)	0.14±0.09	0.43±0.06	0.31±0.08	0.26±0.04
Control	0.02±0.04	0.47±0.07	0.07±0.01	0.23±0.05
p-value	1.6E-05	0.05	2.1E-05	0.29

Table 1 Area of plaque and lumen (mean ± SD) measured by MRI and histology.

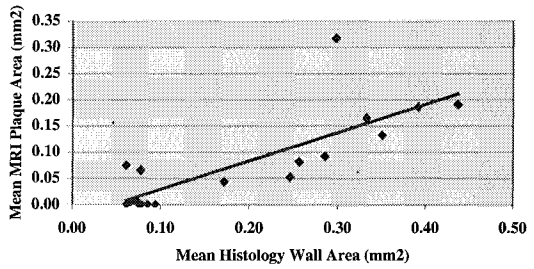

Figure 2 Correlation between mean plaque area measured by MRI and histology (R^2 = 0.64).

References

1. Tunick,P.A. and Kronzon,I. *J.Am.Coll.Cardiol.* 35, 545 (2000).
2. Johnson,J.L. and Jackson,C.L. *Atherosclerosis* 154, 399 (2001).
3. Fayad,Z.A., *et al. Circulation* 98, 1541 (1998).
4. Hockings,P.D., *et al. Proc.ISMRM* 7, 86 (1999).