# High Resolution Magnetic Resonance Imaging of Excised Atherosclerotic Carotid Tissue: The Effects of Specimen Temperature on Image Contrast

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# ABSTRACT

Previous Magnetic Resonance (MR) studies of carotid endarterectomy (CEA) specimens have been directed at interpreting image contrast to determine plaque composition and stability. Such studies may give misleading results since it is believed that the acquired MR data is affected by the conditions used to store the excised tissue. This has been investigated in a high-resolution imaging study of the changes in contrast occurring with a change in specimen temperature. MR images were acquired from 20 CEA specimens. The initial MR examination was made within four hours of excision in tissue maintained and imaged at body temperature. Specimens were subsequently cooled and then re-examined at different times. The MR data was interpreted by comparison with histology obtained from equivalent sections. With the exception of signals arising from blood, changes in MR image contrast occurring in the 24-hour period after the CEA were relatively slight. Plaque lipid T2-weighted image intensity was initially relatively hyperintense in some samples and decreased with time on cooling reflecting a decrease in the lipid transverse (T2) relaxation time. High-resolution imaging allowed a detailed description of plaque calcification and showed that clot retraction was probably responsible for the marked change in the distribution of signals assigned to intraluminal blood. The use of high-resolution MR imaging to examine freshly excised specimens at body temperature showed previously unreported image features. This is important in the design of in vivo MR studies directed at assessing plaque stability by identifying the composition of the atheromatous tissue.

## INTRODUCTION

Magnetic Resonance (MR) imaging is currently the leading non-invasive imaging technique for assessing soft tissue and has the potential to identify plaque constituents and thereby patients at risk from stroke (1). Previous studies involving MR imaging and histology (2–6) have examined excised atherosclerotic

Received 23 May 2007; accepted 30 August 2007. Keywords: Atherosclerosis, Carotid Arteries, High-Resolution In-Vitro Magnetic Resonance Imaging. The authors are grateful to The Health Foundation for a grant (2312/1127) that funded this study. Correspondence to: Dr Steven F. Tanner tel: 0044-113-3438325 fax: 0044-113-3438328 e-mail: sft@medphysics.leeds.ac.uk plaques with the aim of determining the relationship between plaque composition and MR image contrast. There have been reports suggesting that the MR image contrast obtained from excised plaque is affected by storage conditions, particularly in T2-weighted images obtained from lipid (4, 5, 7–10). Previous reports involving carotid endarterectomy (CEA) specimens have either analyzed the tissue at body temperature or they have used high resolution imaging techniques (7, 11–12). We believe that the examination protocol used in this study is the first that combines both high resolution imaging with measurements made at body temperature in a study of intact human CEA tissue made within a few hours of tissue excision. Our imaging study used a clinical scanner to facilitate the comparison of image data with future in vivo MR studies. Scanning CEA specimens within hours of excision and at body temperature may not be practical in many centers. A subsidiary aim of this study, therefore, was to assess changes occurring within 24 hours of excision in specimens stored at room temperature.

#### METHODS

Twenty patients (median age 72 years-interquartile range 67-82 years; 17 male) with stenotic atherosclerotic disease of at least one carotid artery were recruited consecutively. All patients gave informed written consent for the study, which had received prior approval from the local research ethics committee. Every patient had an internal carotid artery stenosis of at least 70%. Seventeen patients were symptomatic (transient ischaemic attack [n = 10], amaurosis fugax [n = 4] and stroke [n = 3]) whilst 3 patients were asymptomatic. Carotid endarterectomy (CEA) was performed using a method that yielded intact plaques 20-35 mm long (13). Immediately after resection, the CEA specimens were placed inside an incubator and maintained at body temperature and 100% Relative Humidity (RH). MR data was acquired both from the excised tissue and from an adjacent sealed 'phantom' containing vegetable oil and placed within the imaging field of view. This phantom provided a reference signal allowing the evaluation of any changes in shape or signal intensity associated with the excised specimen.

The initial MR examination was made within 4 hours of the CEA in most specimens and employed a device that circulated warm air around a sealed compartment in which the excised tissue was maintained at 37°C and 100% RH (14). The specimens were then allowed to cool to room temperature (approximately 20°C) before re-examination 18–24 hours later. In 13 samples, a further MR measurement was made shortly after the initial examination and just after the specimens had cooled. These samples were left in the scanner before their final MR examination thereby allowing the assessment of any shape changes occurring in the ~24-hour period after resection. Two specimens were refrigerated at 4°C for 5 days and were examined both before and after the storage period using either water- or lipid-proton selective imaging or fat-suppressed imaging techniques.

MR data was obtained using a 1.5T Gyroscan ACS-NT whole-body scanner (Philips Medical Systems, Best, the Netherlands) equipped with a 23 mm diameter surface MR microscopy receiver coil. A 45 mm Field of View was used together with a 1 mm slice thickness and an acquisition matrix of  $256 \times$ 256 reconstructed to  $512 \times 512$ . The resulting image resolution was therefore approximately  $180 \times 180 \times 1000 \ \mu m$  although interpolation gave displayed images with voxel dimensions of  $90 \times 90 \times 1000 \mu$ m. T1-, T2-, and proton density-weighted spin-echo images were acquired using repetition times (TR) and echo-times (TE) of 475 ms/16 ms, 3500 ms/100 ms and 2000 ms/16 ms, respectively. Images with acceptable signal to noise ratios were obtained by employing signal averaging techniques. The T1-, T2- and proton density-weighted data sets were acquired using 8, 12 and 8 acquisitions, respectively, in times of between approximately 15 minutes for the proton density- and 17 minutes for the T2-weighted images.

After MR examination, the specimens were fixed in a buffered formaldehyde solution and then decalcified using EthyleneDiamineTetraAcetic Acid (EDTA). Five micron sections were obtained from transverse sections of the plaque at 2 mm intervals and stained using Haematoxylin and Eosin (H&E), Miller's Elastic and Sirius Red. Images of these sections were then assessed and the following features graded on a five-point scale: degree of stenosis, calcification, lipid core, inflammation (acute or chronic), hemorrhage (recent or old), thrombus (recent or organizing), neovascularisation (extent), plaque cap ('thick or thin') and plaque rupture (recent or re-stabilizing). Caps were deemed 'thin' if an intact endothelium was supported by a continuous layer of 2 to 3 collagen and elastic fibrils above a lipid core.

Plaque was considered unstable when the histological boundary between the arterial lumen and the established plaque did not contain elastic and collagen fibres. All recently ruptured plaques were considered unstable because of a continued propensity for thrombus formation or contents discharge. 'Recent' rupture of a plaque was determined by cavitation of a plaque accompanied by distinctive 'lips' at the margin of excavation, local intraplaque haemorrhage, fibrin deposition and attempted repair by re-endothelialisation from the cavity margins with or without attempted organization of plaque contents. 'Old' rupture presented as cavitation of the elastic fibre contour of the artery with 'lips' to a stabilized, completely endothelial-lined cavity.

The MR and histology sections were matched using the known distances of the MR slice from the excised carotid bifurcation and by comparison of image morphology (5, 10). MR image contrast was then interpreted from the histology.

## RESULTS

# Histology

Microscopy confirmed severe restriction of the arterial lumen in all specimens (with occlusion on one case) necessitating the surgical intervention. All carotid endarterectomy (CEA) specimens showed complex plaques encompassing AHA class V through VI with subsets (15) and end-stage VII lesions (16). Stenosis commenced in the common carotid artery and extended through the bifurcation into the internal carotid artery. Of the 20 specimens studied, 9 lipid-rich plaques were considered unstable, 2 lipid-rich plaques appeared stable, and the 9 remaining plaques were stable with a variable degree of calcification. In 6 of the 9 unstable plaques, recent hemorrhage was detected, and 8 were ruptured with 3 of the 8 showing attempted repair of the rupture.

#### MR Imaging-Specimen shape

In 17 of the 20 CEA specimens examined, the lumen boundary showed an irregular contour, suggesting bulging of tissue into the lumen or a rounded indentation into the atheroma (Fig. 1). In 3 samples, a marked cavity was observed within the MR data arising from plaque rupture with evacuation of lipid and other debris. An example is displayed in Fig. 2 showing re-endothelialization of the margin of the rupture and part of the cavity. A slight change in overall sample shape was detected in 6 of the 13 specimens that were left in the scanner. This occurred predominantly towards the ends of the specimens where calcification was less prevalent.



darterectomy specimens acquired from two different patients showing irregular lumen contours arising from the bulging of tissue into the lumen or an indentation of the atheroma. These images also show evidence of discrete calcified nodules (arrows) (a) and a band of calcification (arrow) (b). Calcification consists of signal hypointensity in T1-, T2- and proton density-weighted MR images and was identified from histology sections obtained from the equivalent slice (histology data not shown).

#### MR Imaging-Specimen signal intensities

Typically the excised carotid specimens showed areas of calcification, intra-plaque hemorrhage, pooled blood, fibrous and elastic tissue and lipid.

All 20 atheromatous plaques examined contained some calcification represented by regions of hypointensity in all of the acquired MR data. This often occurred as an 'egg-shell' band at the periphery of the plaque but could also be encountered as discrete nodules within the atheroma (Fig. 1) and in one specimen as a distinct lamina in an otherwise fibroelastic cap (Fig. 3b). In one severely stenotic, heavily calcified plaque, part of the restriction was due to an almost pedunculated calcified nodule, involving the distal common carotid and merging with the narrowed bifurcation (Fig. 3d).

In 16 of the 20 specimens examined, MR data was acquired showing signals that could be attributed either to intra-plaque hemorrhage, blood within a cavity resulting from plaque rupture, residual blood within the vessel lumen or blood within tissue clefts on the outer aspect of the sample.



**Figure 2.** H and E stained section (a) and corresponding T1weighted MR image (b) of a section through an excised internal carotid specimen. The histology section shows a blood clot within a cavity created by the plaque rupture (arrow). There are also areas of calcification (solid arrow) and hemorrhage into lipid (star).



**Figure 3.** H and E stained sections (a and c) and Proton Densityweighted MR images (b and d) obtained from CEA specimens resected from two different patients. Figs. (a) and (b) were acquired from the internal carotid artery and show a thin calcified layer (arrowed) within the fibroelastic cap. Figs. (c) and (d) were obtained from a different specimen and show a very large calcified almost pedunculate atheromatous nodule (arrowed) occupying a significant part of the lumen within the common carotid artery. The thin band of high signal intensity separating nodule and lumen in (d) is likely to arise from blood clot adhering to the surface of the nodule.

Signal intensity arising from bleeds into the plaque were detected in 14 specimens and in all 9 plaques classified as being unstable. These bleeds were confirmed by histology and gave variable T2-weighted signal intensity and a high signal on T1weighted images that was only slightly less intense than that associated with the adjacent vegetable oil phantom (17).

Intraluminal-blood or clot was identified in the MR image data by its location, signal magnitude and changes occurring in signal intensity with time. Blood gave rise to high signal intensity within the lumen in both Proton Density- and T2-weighted images acquired immediately after CEA. During the next 24 hours, there were often marked changes in the distribution of this high signal that were not due to changes in specimen or lumen shape noted previously. Reposition of high signal from intraluminal and intracavity blood independent of gravity was encountered. In one instance a fluid-fluid boundary was observed (Figs. 4a and b) that separated a lower layer showing a relatively high signal on T1- and relatively low signal intensity on T2-weighted images. When this sample was re-examined approximately 21 hours after endarterectomy, the layering was less evident (Figs. 4c and d).

The signal intensity observed in the T1-weighted images arising from the fibrous and elastic tissue was relatively low when compared with the signal returned by the adjacent vegetable oil phantom. In contrast, this tissue gave high signals in the T2and proton density-weighted images that were only slightly less intense than that emanating from the phantom (data not shown).

Because of the variable signal associated with hemorrhage, a detailed assessment of changes in lipid signal intensity was only made in 4 specimens where histology showed no evidence





of intra-plaque hemorrhage and where lipid could be identified. In initial scans lipid gave relatively high signals in the T1- and T2-weighted images (Fig. 5b). On cooling to room temperature for a period of approximately 24 hours, a slight decrease in lipid signal intensity was observed in the T2-weighted data (Fig. 5c). A reduction of about 20–25% in signal was observed in the data shown in Fig. 5. In the two specimens stored at 4°C for 5 days, there was a marked loss of T2-weighted lipid signal intensity (whilst proton density signal was unaffected).

# DISCUSSION

This study did not seek to fully characterise CEA specimens using methods based solely on MR imaging. Shinnar et al. (5) have shown that this would require the acquisition of data using more imaging sequences than those employed here. Our aims were to improve the understanding of MR image contrast in atherosclerotic plaque using excised tissue maintained in conditions that are close to those found in-vivo and then to assess short-term changes occurring with specimen cooling. The results indicate that, with the exception of signals arising from blood, only minor changes in tissue contrast occurred in the acquired T1-, T2- and proton density-weighted MR images.



**Figure 5.** H and E stained (histology) (a) and corresponding T2weighted MR images (b) and (c) of a section through the common carotid artery. The data shown in Fig. 5b was acquired 4 hours after resection with the specimen maintained and scanned at body temperature. Fig. 5c was obtained after the specimen had been kept at room temperature for approximately 21 hours after tissue resection. The arrows show an area identified from the histology as lipid within this section. Comparison with signals arising from the phantom (not shown) indicate that a decrease in signal intensity has occurred within this area after resection and specimen cooling that is consistent with a decrease in the T2 relaxation time.

All specimens examined had some blood present both on the surface and within the lumen. A deliberate choice was made to examine tissue without washing off this blood or to carry out any other form of sample processing. This avoided the detection of MR signals from the 'washing medium' and prevented the mechanical loss of any friable intraluminal material (e.g., thrombus, necrotic debris, etc.). It is known that changes occurring in blood can affect the spin-echo MR signal intensities (18). It was not possible to transect intact specimens and examine the nature and distribution of blood during a study involving repeat MR examinations. We can, therefore, only speculate on the interpretation of the spin-echo MR signal intensities arising from the intraluminal blood. Changes in the distribution of signals assigned to intra-luminal/cavity blood were often observed in our study. These changes were not simply a consequence of gravity or due to small changes in sample shape but probably reflected the process of clot retraction whereby the clot expresses serum whilst compacting the red cell component. The fluid-fluid boundary shown in Fig. 4 is similar to that reported previously in an MR study of cerebral intra-ventricular hemorrhage (19). This 'layering pattern' is consistent with a change in protein concentration resulting from precipitation of the red blood cells. After 24 hours, the clear demarcation between layers (Figs. 4c and d) was no longer as marked, possibly as a result of clot retraction.

Most specimens showed evidence of intra-plaque haemorrhage that gave MR signal intensity in good agreement with a previous study that classified such bleeds as 'fresh', 'recent' or 'old' (17). The signal arising from blood is affected by a number of factors, including the integrity of the red blood cells and the oxidation state associated with the iron in hemoglobin (19). Where histology showed only intact red blood cells within the plaque, then the bleed was considered to be 'fresh' and the MR images showed relatively high T1- and low T2-weighted signal intensities. Where lysed red cells, mixed or layered with intact red cells, fibrin and interspersed endothelial cells (organization) were found, a bleed was deemed 'recent' (i.e., pre-operative) and relative hyperintensity was observed in both the T1- and T2-weighted data.

Assessment of change in the lipid signal was restricted to specimens where histology showed the absence of hemorrhage. This allowed any change in T2-weighted signal intensity to be assessed without the complication of a changing signal associated with the blood. The study showed a relatively high signal arising from lipid in the T2-weighted MR data. This image intensity decreased slightly over a period of approximately 24 hours when these specimens were cooled to room temperature (Fig. 5) with a more marked loss in signal occurring in the two specimens stored at 4°C for 5 days. Whilst similar high signals have been observed previously (4), our observation is not consistent with some previous studies that have assigned a relatively short T2 to lipid. This can be accounted for if the study by Berr et al. (9) is considered where it is suggested that misleading signals may arise as a consequence of a lipid phase change occurring at temperatures below 37°C. At body temperature, the lipid is in the isotropic phase, (with a relatively long T2 value) changing to the smectic liquid crystalline or solid phase (with a shorter T2) on cooling. Our results are consistent with this interpretation and, as far as we are aware, are the first to follow changes occurring within the same CEA specimen. It should be noted that most subjects recruited for this study had established atherosclerotic disease. There was therefore bias in our selected 'specimen cohort.' Our study examined specimens harvested from patients with relatively advanced (older) atheromas. This population is expected to have higher amounts of cholesteryl esters and, hence, cholesterol monohydrate (9). It is possible, therefore, that some of the lipid within even freshly excised specimens may have contained some lipid rich necrotic core tissue characterized by a relatively shorter value of T2. The identification of lipid signals may be further complicated because an in vitro study has shown that the lipid signal can be heterogeneous depending not only on the composition of the lipid constituents but also on their physical state and mixing (20).

The high resolution MR imaging techniques employed allowed the detection of very small areas of calcification. Thus, in one specimen, calcification could even be detected in the plaque cap (Fig. 3b). In most excised tissue, larger areas of calcification were observed occurring either as nodules dispersed throughout the plaque or as a thin 'egg-shell' band at the outer margin of an atheroma (Fig. 1). The large heavily calcified nodule observed in Fig. 3c occupied approximately half of the lumen and probably represents old calcified thrombus. The low signals returned by both calcification and lumen make distinction between them impossible when using MR data alone (Fig. 3d). It is of interest to note that *in-vivo* studies of the carotids employing only 'black-blood' MR imaging techniques are unlikely to reveal such a stenosis.

#### Summary

The results of this high resolution MR imaging study of intact CEA specimens has shown only slight changes in image contrast occurring when specimens were cooled from body-temperature and then maintained at room-temperature for a period of  $\sim 24$  hours. This information is of value in the design of studies carried out in centres where excised tissue cannot be examined at body temperature and where scanner availability is constrained by clinical imperatives. The study has also followed the change in lipid contrast occurring with specimen cooling suggested in some previous reports. It has shown how blood can give rise to highly variable signal intensity even when pooled in freshly excised specimens. Further, the high-resolution imaging techniques employed have allowed a detailed description of the patterns of calcification occurring within carotid plaque. These results are important for the interpretation of corresponding *in vivo* data acquired with the aim of assessing plaque stability.

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