

## T2 mapping MRI technique quantifies carotid plaque lipid, and its depletion after statin initiation, following acute myocardial infarction

Mohammad Alkhalil<sup>a,b</sup>, Luca Biasioli<sup>a</sup>, Naveed Akbar<sup>b</sup>, Francesca Galassi<sup>a</sup>, Joshua T. Chai<sup>a</sup>, Matthew D. Robson<sup>a</sup>, Robin P. Choudhury<sup>a,b,\*</sup>

<sup>a</sup> Acute Vascular Imaging Centre, Radcliffe Department of Medicine, University of Oxford, UK

<sup>b</sup> Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

### HIGHLIGHTS

- T2 mapping is a newly-validated magnetic resonance (MRI) technique that can quantify carotid lipid content in patients who were not pre-screened for carotid atherosclerosis.
- T2 mapping can detect reduction in carotid lipid content following three months of statin treatment.
- This reduction in plaque lipid was not related to blood lipid measurements suggesting that these measurements are not substitutes for quantifying plaque lipid.
- These findings support the use of plaque imaging to identify patients who may be suitable for intensive lipid treatments.

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### ABSTRACT

**Background & aims:** A recently-validated, highly-sensitive T2 mapping magnetic resonance (MRI) technique accurately quantifies carotid plaque lipid. The aims of this study were to determine: (i) the extent of carotid plaque lipid in patients with acute coronary syndromes (ACS); (ii) the effects of initiation of high-intensity statin on plaque lipid content and (iii) whether plaque lipid content is related to standard or 'functional' blood lipid measurements.

**Methods:** Statin naïve subjects presenting with ACS underwent carotid artery MRI at 3 T scanner to quantify plaque lipid. Patients were subsequently commenced on high dose statin as part of clinical care and underwent a second MRI after three months. Plaque composition was measured using objective semi-automated techniques. **Results:** 23 out of 24 patients had measurable lipid. Three months after statin initiation there was a significant reduction in carotid lipid percentage [from 10.3% (7.2–14.2) to 7.4% (5.4–10.0),  $p = 0.002$ ] and a significant increase in fibrous percentage [from 83.3%  $\pm$  6.6–85.5%  $\pm$  4.8,  $p = 0.039$ ]. None of the studied functional blood biomarkers were related to either baseline carotid plaque lipid content or its propensity to change with statin treatment.

**Conclusions:** T2-mapping demonstrated depleted carotid plaque lipid following the initiation of high-intensity statin treatment. Standard or 'functional' blood biomarkers were dissociated from plaque lipid content or changes with treatment. These findings further reinforce the importance of disease characterisation over risk factor assessment. Subject to clinical trial findings, quantification of plaque lipid may provide the basis for an approach to identify patients suitable for intensive lipid reduction regimes.

### 1. Introduction

Low-density lipoprotein cholesterol (LDL-c) reduction, especially with statins, has been consistently effective in reducing cardiovascular events [1]. The effects of LDL-c reduction have been further reinforced

by the outcome trials of ezetimibe and evolocumab [2,3], in which additional LDL-c reduction was associated with a further reduction in cardiovascular events. Remarkably, it is still unclear precisely *how* reducing LDL-c translates into improved cardiovascular outcomes. One prevalent theory is that LDL-c reduction leads to a net cholesterol

\* Corresponding author. Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, United Kingdom.

E-mail address: [robin.choudhury@cardiov.ox.ac.uk](mailto:robin.choudhury@cardiov.ox.ac.uk) (R.P. Choudhury).

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evacuation from atherosclerotic plaque and that this, in turn, has a 'stabilising effect' on plaques, which are then less likely to precipitate acute events [4].

In experimental atherosclerosis, reduction in plasma LDL-c has been associated with depletion of plaque lipid content and changes in plaque composition [5,6]. Similarly, *ex-vivo* analysis of explanted carotid endarterectomy specimens in patients treated with statins has shown depletion in lipid core, with an increase in collagen content compared to placebo [7]. Further advances in non-invasive imaging modalities have allowed the study of lipid-lowering drugs on plaque composition, *in vivo*. [8–10] However, these studies have been fundamentally limited by the inherent insensitivity of imaging tools [4,11]. Multi-contrast (MC-MRI) relied on patients with sufficiently large areas of coalesced lipid to be discerned, meaning that only a minority of patients have sufficient lipid to be quantified serially [4,12]. Because of the previous requirement for large lipid cores, earlier studies have often focused on patients with pre-identified carotid atherosclerosis [8,9]. Such limitations with imaging tools have rendered studying any relationship with blood lipid biomarkers imprecise. In fact, previous studies reported poor and insignificant correlation between plaque lipid and blood lipid biomarkers [8]. However, new functional blood lipid biomarkers have recently emerged and have added more insights into the clinical risk of atherosclerosis. HDL functionality is a novel marker of HDL quality which can be assessed using different methodologies [13]. Cholesterol efflux capacity acts as a surrogate of reverse cholesterol transfer and as a reflective of the function of HDL [14,15]. This biomarker has been shown to predict clinical outcomes even after adjusting for HDL-c (cholesterol) [14,15]. Nonetheless, HDL particle number was found to be superior to HDL functionality or HDL-c in predicting incidence of cardiovascular disease [16]. This may explain why HDL-c failed to predict prognosis in secondary prevention patients with low LDL-c [17].

T2 mapping is a newly-validated MRI technique that accurately identifies plaque lipid, at a voxel by voxel level based on its physical properties [4,12,18,19]. This technique has also enabled investigation of the relationship between lipid *distribution* and the symptomatic status in the carotid arteries, beyond the volumetric quantification of vessel lipid content [18].

Therefore, we reasoned that the ability to quantify vessel lipid content with high sensitivity may allow the effects of intensive LDL-c reduction to be quantified serially with much greater precision than has been previously possible and in patients who do not necessarily have established carotid disease, or large lipid-rich necrotic cores. Furthermore, we hypothesized that since measures of blood cholesterol vary relatively little during adult life, while atherosclerosis accumulates progressively, there would be no meaningful relationship between blood lipoprotein measurements and plaque [lipid] volume. Such a finding would further emphasize the importance patient characterisation over risk factor assessment.

In this proof-of-concept study we used T2 mapping MRI to determine: (i) the extent of carotid plaque lipid in patients with acute coronary syndromes; (ii) whether plaque lipid content is related to a range of 'functional' lipid measures and (iii) the effects of initiation of high-intensity statin on plaque lipid content.

## 2. Patients and methods

### 2.1. Study population

*Statin naïve* patients admitted with an acute coronary syndrome (ACS) to the Oxford University Hospital NHS trust were recruited between April 2016 and November 2016. ACS was defined according to the third universal definition of myocardial infarction [20]. In detail, patients presenting with ischemic-type chest pain, associated with electrocardiogram (ECG) changes and/or elevated cardiac troponin and scheduled for invasive coronary angiography were recruited. Eligible patients, with no MRI contraindication, were scanned at the Oxford

Acute Vascular Imaging Centre (AVIC) within 48 h of their admission. Recruited patients were all adults (> 18 years of age) who had not been exposed to any form of lipid-lowering drugs, including statins, at the time of admission. Patients were commenced on intensive statin therapy (daily atorvastatin 80 mg) as part of routine clinical care [21], and were scheduled for a second MRI scan after three months.

Ethical approval was obtained from National Research Ethics Services (NRES) and Oxford R&D committee prior to commencement of the study. All patients provided written informed consent prior to participation in this study.

### 2.2. MRI protocol

Both carotid arteries were studied at 3T (Verio, Siemens Healthcare, Erlangen, Germany) with a 4-channel phased-array carotid coil (Machnet, Roden, The Netherlands) or 10-channel phased-array carotid coil (Pulseteq, Surrey, UK). Bright-blood, time-of-flight (TOF) angiography of the carotid arteries was acquired to localize carotid bifurcation and atherosclerotic plaque. Multi-slice carotid T2 maps were generated from 14 images with echo times  $TE = 9\text{--}127$  ms and repetition time  $TR = 2$  s as previously described [18,19]. Ten slices of 2 mm thickness each, covering 2 cm of both carotid arteries were acquired. Slices were centralised on the maximum plaque volume identified on TOF and T1 weighted images so full plaque length was covered within 10 slices. All follow up MRI scans were obtained using identical sequence protocols and were matched, with baseline MRI scans, using carotid bifurcation as an internal landmark. A standardised patient position with shoulders pulled-down and full neck extension was coupled with a customised neck brace to minimise movement. Both left and right carotid arteries were matched separately to account for any subtle difference in neck position between baseline and follow up.

### 2.3. Data analysis

T2 maps of the carotid arteries were generated *voxel-by-voxel* using mono-exponential nonlinear fitting [12], and lumen and external vessel boundaries were segmented using a validated semi-automated procedure [22]. A segmentation method to identify plaque components was implemented using T2 threshold less than 42 ms for lipid and T2 threshold more than 42 ms but less than 90 ms for fibrous tissue, as previously validated [12,19]. Plaque components were quantified using a single voxel as one unit (spatial resolution of  $0.33 \times 0.33 \times 2$  mm) and voxels, according to T2 thresholds, were aggregated for each vessel, to yield lipid volume and fibrous volume. Lipid and fibrous volumes of the studied vessel were expressed as the average lipid and fibrous volume obtained from all of the 10 studied slices and irrespective of the degree of stenosis. Vessel wall volume was calculated from the difference between external vessel boundary and vessel lumen. In keeping with previous studies of carotid atherosclerosis, a mean value was obtained for left and right carotid arteries and was used per subject for analysis [23,24]. All algorithms were implemented in Matlab (MathWorks, Natick, USA).

### 2.4. Blood lipid biomarkers assays

Serum and EDTA-plasma were centrifuged for 15 min at 4°C within 1 h of blood collection and stored in aliquots at  $-80^{\circ}\text{C}$  for future analyses. Standard lipid measurements (i.e. LDL-c, total cholesterol, HDL-c and triglyceride) in addition to lipoproteins particle size and concentrations were analysed using nuclear magnetic resonance (NMR) spectroscopy using Axion lipofIT-S100 (Numares AG, Regensburg, Germany). ApoA1, high sensitive CRP (hs-CRP) and PCSK9 were determined in plasma by ELISA using kits from R&D systems Ltd (Abingdon, UK). Cholesteryl ester transfer protein (CETP) activity was measured using a fluorometric method using assay kits from Abcam (Cambridge, UK). Lipoprotein (a) was measured in serum using ELISA

kit from Abcam (Cambridge, UK). The cholesterol efflux capacity of HDL was determined using fluorescence-labelled cholesterol method as validated previously [25].

### 2.5. Sample size calculation

Previously published data using T2 mapping demonstrated that lipid was present in carotid arteries irrespective of the symptomatic status or degree of luminal stenosis [18]. Based on a previous report to determine sample size in studying treatment effect using MRI-derived carotid atherosclerosis as primary endpoint and assuming that statins have a modest effect of 20% reduction in lipid content within three months [7,26], sample size of 20 patients was required for 80% power and 2-sided level of 0.05. Total sample size was estimated to be 22 allowing for 10% drop out of patients.

### 2.6. Statistical analysis

The Shapiro-Wilk test was used to assess whether data were normally distributed. Data are presented as mean  $\pm$  standard deviation or median and interquartile range, as appropriate. Measurements at each time point were compared using paired *t*-test for normally distributed data or Wilcoxon signed-rank test for non-normally distributed data. Categorical data between response groups were analysed by the  $\chi^2$  test, while continuous variables were assessed with unpaired *t*-test or Mann Whitney test, where appropriate. Correlations were assessed using Spearman rank test. Statistical analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, USA) and *p*-values  $< 0.05$  were considered statistically significant.

## 3. Results

A total of 27 patients were recruited during the study period. Three patients were excluded due to inadequate MRI image quality. Three patients who did not complete their follow up scan were also excluded from the analysis. Of the remaining 21 patients, 11 patients had presented with ST-segment elevation myocardial infarction (STEMI) and 10 patients with NSTEMI. The mean ( $\pm$  SD) age was  $60 \pm 10$  years with two-thirds of patients male. Clinical and lipid characteristics are summarized in Table 1.

Reproducibility was performed on almost 25% of analyzable data ( $> 20$  vessels) randomly selected and irrespective of plaque burden, lipid percentage, image quality or timing of the scan (baseline or follow up). Intra-observer variability was 3.15% with interclass correlation of 0.997 (95% confidence interval, 0.994–0.999,  $p < 0.001$ ) and measurement of error (defined as the absolute difference in lipid percentage) of  $< 1\%$ .

Most patients had measurable carotid lipid, with 23 out of 24 patients having lipid percentage more than 1%. There was a significant correlation between left and right carotid lipid content ( $r = 0.53$ ,  $p = 0.016$ ).

### 3.1. Changes in plaque composition

After a median follow up of 107 days (IQR 100–137) after initiation of intensive statin treatment, there was significant reduction in carotid vessel lipid percentage (lipid%) from 10.3% (7.2–14.2) to 7.4% (5.4–10.0) ( $p = 0.002$ ) (Figs. 1 and 2). Similarly, there was a significant reduction in vessel lipid volume from  $4.7 \text{ mm}^3$  (2.7–6.3) to  $3.4 \text{ mm}^3$  (2.1–4.5) ( $p < 0.001$ ).

Compared to baseline, there was significant increase in carotid vessel fibrous percentage (fibrous%) following intensive statin treatment from  $[83.3\% \pm 6.6\text{--}85.5\% \pm 4.8]$ ,  $p = 0.039$ ] (Figs. 1 and 2). There was no significant change in fibrous volume over the study period [ $36.6 \text{ mm}^3$  (31.4–47.4) versus  $37.4 \text{ mm}^3$  (31.7–42.4),  $p = 0.079$ ].

**Table 1**

Baseline clinical and lipid characteristics of recruited patients.

Patients characteristics, N = 21		Mean $\pm$ SD
Age		64 $\pm$ 10
Male (%)		15 (71%)
Body mass index		29 $\pm$ 5
Cardiovascular risk factors	Hypertension	8 (38%)
	Diabetes	1 (5%)
	Active smoking	8 (38%)
	Family history of IHD*	6 (29%)
Medication on admission	Antiplatelet	3 (14%)
	Beta or calcium blockers	2 (10%)
	ACE or ARB*	7 (33%)
	Anticoagulation	0 (0%)
Presentation	NSTEMI	10 (48%)
	STEMI	11 (52%)
Troponin (ng/mL)		17 $\pm$ 21
LDL-c (mg/dL)		109 $\pm$ 30
Total cholesterol (mg/dL)		176 $\pm$ 38
HDL-c (mg/dL)		46 $\pm$ 11
Triglyceride (mg/dL)		151 $\pm$ 56
Lipoprotein particle size (nm)	LDL-s	20.9 $\pm$ 0.4
	HDL-s	8.8 $\pm$ 0.5
	VLDL-s	48.9 $\pm$ 5.5
Lipoprotein particle number (nmol/L)	LDL-p	1389 $\pm$ 452
	HDL-p	31977 $\pm$ 5529
	VLDL-p	4.82 $\pm$ 5.88
Lp(a) (mg/dL)		10.2 $\pm$ 6.2
ApoA1 (mg/dL)		176 $\pm$ 52
hsCRP (mg/L)		7.2 $\pm$ 3.6
PCSK9 (ng/ml)		278 $\pm$ 94
Cholesterol efflux capacity (%)		23 $\pm$ 3
CETP activity (nmol/mL per hour)		17 $\pm$ 2

### 3.2. Relation between carotid lipid content and functional blood lipid markers

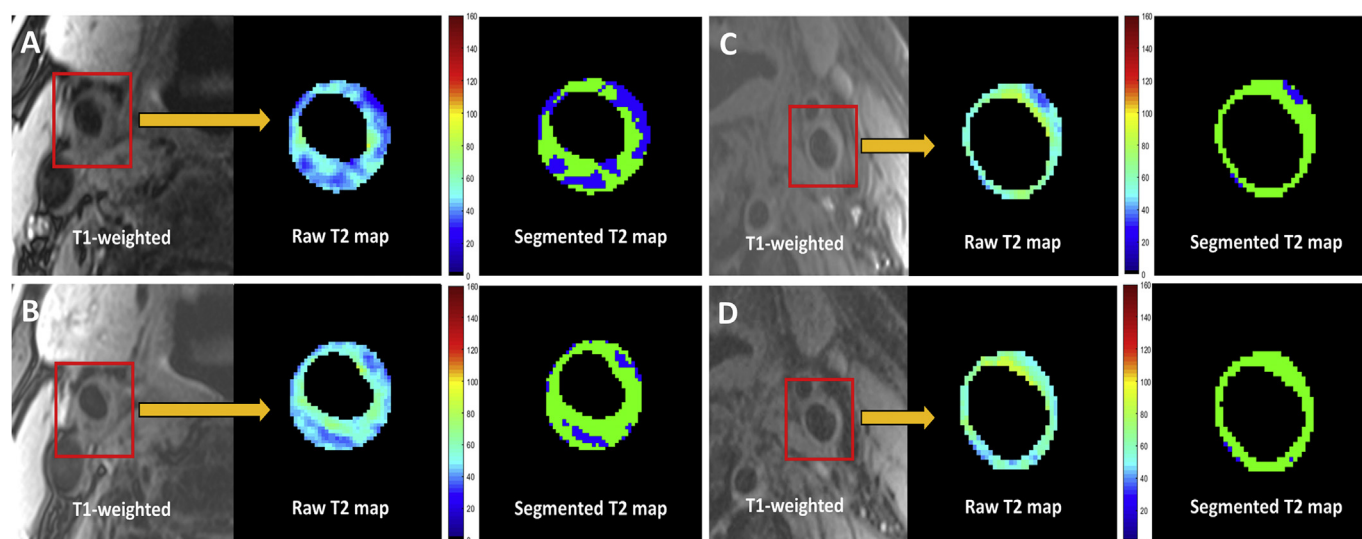
We compared plaque lipid content to conventional plasma lipoprotein measurements (total cholesterol, LDL-c, HDL-c & triglycerides) and no significant correlation with *baseline* lipid% was identified. We then examined the size and concentrations properties of plasma lipoprotein particles and, similarly, found no association with *baseline* lipid %. We next explored whether emerging functional measures (Lp(a), PCSK9, CETP activity and cholesterol efflux capacity) were related to carotid lipid%. Likewise there was no association when compared with *baseline* carotid lipid%. The observed coefficients ranged between  $-0.34$  and  $0.35$  across all standard and functional blood lipid biomarkers.

None of the standard or functional blood lipid biomarkers showed any relationship to *change* in carotid lipid%. This was assessed using *baseline* and *follow up* blood measurements.

Similarly, with the exception of hs-CRP, there were dissociations between *change* in carotid lipid% and *change* in both standard and functional blood lipid biomarkers. *Change* in hs-CRP showed a modest relationship with *change* in carotid lipid% ( $r = 0.46$ ,  $p = 0.038$ ).

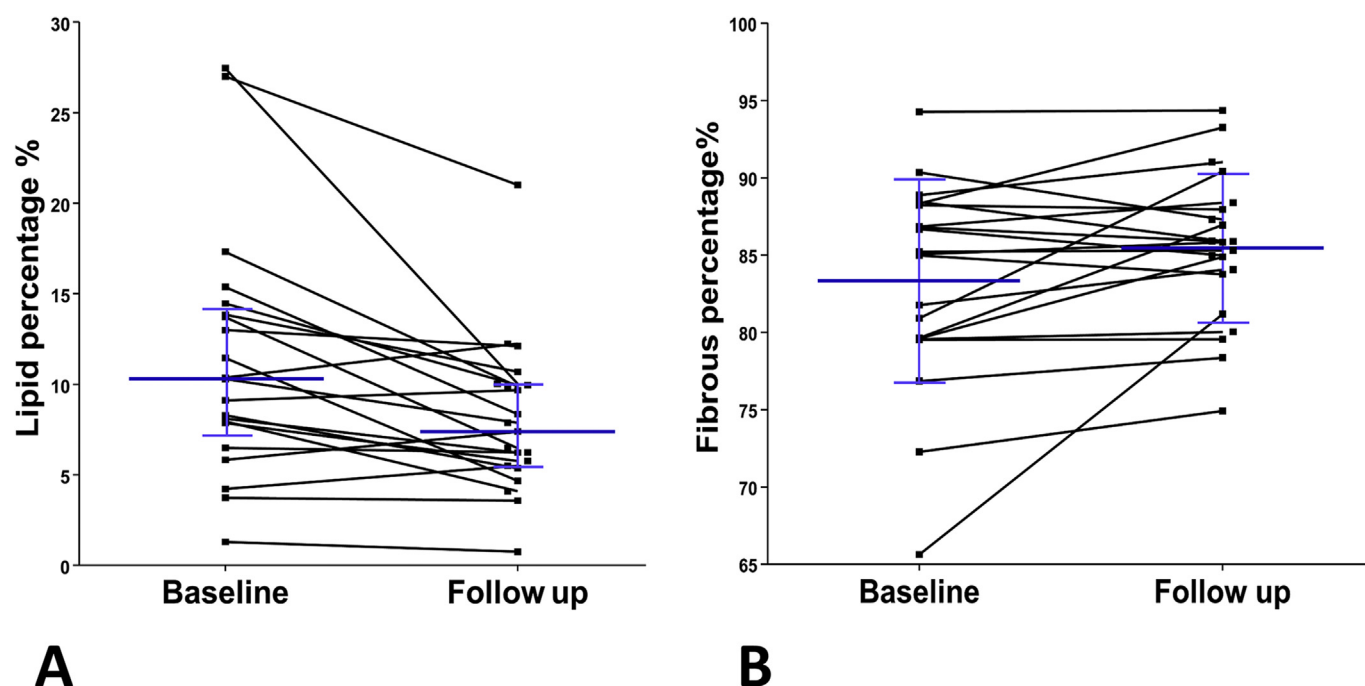
### 3.3. Blood lipid biomarkers between patients according to magnitude of carotid lipid% reduction

Using median reduction in carotid lipid% as cut-off ( $-2.4\%$ ), blood lipid biomarkers were evaluated between patients who “responded” and “did not respond” to statin treatment (reduction in lipid %  $>$  or  $\leq$  median). Carotid lipid% at baseline was significantly higher in the ‘responders’ group compared to the ‘non-responders’ group ( $13.8\%$  versus  $7.2\%$ ,  $p = 0.002$ ) (Table 2). *Baseline* and *change* over time in both standard and functional lipid biomarkers were comparable between the two groups, with the exception of *change* in hs-CRP, which was significantly larger in magnitude in the ‘responders’ group.



**Fig. 1.** Changes in carotid plaque composition on T2 mapping.

(A) Cross-sectional T1-weighted image of right carotid artery plaque from a 63 year old female presented with ACS, with corresponding raw and segmented T2 map images. (B) Significant reduction in lipid percentage from 39% to 15% after 97 days of statin treatment. (C) Cross-sectional slice of left carotid artery from a 72 year old male with ACS, with matching raw and segmented T2 images. (D) Significant reduction in lipid percentage after 111 days from 8.3% to 2.4%.



**Fig. 2.** Carotid plaque composition change over study period.

(A) Significant reduction in carotid lipid% following initiation of high intensity statin. The individual variations of lipid% change with statin therapy suggested that most patients showed some degree of lipid% reduction. (B) Significant increase in carotid fibrous% compared to baseline. The individual variations of fibrous% change with statin therapy demonstrated that majority of patients had fibrous% increase with statin treatment.

### 3.4. Change in plaque lipid content according to the attained plasma LDL-c

When the group was stratified according to the level of attained plasma LDL-c post-intensive statin treatment (above or below 70 mg/dL), there was no significant difference in plaque lipid response between these two groups. This was assessed using *baseline, follow up* and *change* in both lipid percentage and vessel wall volume (Table 3).

## 4. Discussion

The main findings in this study were: (i) Most patients had measurable lipid using a T2 mapping MRI technique, (ii) intensive statin treatment was associated with significant changes in plaque composition detected in ACS patients who were *not screened* for carotid artery disease, (iii) these changes resulted from significant decreases in vessel

**Table 2**

Baseline and change in standard and functional blood lipid biomarkers between responders and non-responders groups.

Variables		Responders	Non-responders	p value
Lipid% change		−6.0 (−7.2, −3.1)	−0.2 (−1.1, 1.3)	< 0.001
Age		62 ± 11	67 ± 8	0.22
Male gender		7 (64%)	8 (80%)	0.64
Imaging parameters	Baseline lipid%	13.8 (10.4, 17.3)	7.2 (4.1, 9.4)	0.002
	Baseline WV	44.8 (41.7, 54.0)	45.4 (33.8, 59.9)	0.83
	Change in WV	−2.8 (−5.6, 1.7)	−1.6 (−8.4, 0.2)	0.62
Standard lipid biomarkers	LDL-c	113 ± 33	105 ± 27	0.55
	Change in LDL-c	−43 (−70, −23)	−30 (−42, −16)	0.16
	TC	179 ± 43	173 ± 33	0.74
	Change in TC	−53 (−97, −26)	−29 (−50, −17)	0.23
	HDL-c	46 ± 13	47 ± 10	0.85
	Change in HDL-c	−4.0 ± 9.5	2.8 ± 8.3	0.10
	TG	146 ± 44	157 ± 69	0.66
	Change in TG	−14 (−106, 17)	−25 (−73, −6)	0.70
Lipoprotein particle size	LDL	20.9 ± 0.3	20.8 ± 0.4	0.63
	Change in LDL	−0.2 (−0.4, 0.2)	0.1 (0.0, 0.28)	0.07
	HDL	8.7 ± 0.4	8.8 ± 0.6	0.54
	Change in HDL	0.9 ± 0.4	0.2 ± 0.2	0.56
	VLDL	47.7 (42.9, 50.2)	48.6 (45.2, 53.9)	0.44
	Change in VLDL	−0.9 (−2.0, 1.5)	1.2 (−2.0, 1.9)	0.70
Lipoprotein particle number	Total LDL	1392 ± 401	1386 ± 524	0.98
	Change in LDL	−666 (−874, −374)	−487 (−797, −198)	0.67
	Large LDL	786 ± 207	810 ± 322	0.84
	Change in L.LDL	−342 (−524, −189)	−290 (−484, −78)	0.75
	Small LDL	622 ± 182	576 ± 314	0.68
	Change in S.LDL	−254 (−349, −71)	−171 (−318, −69)	0.67
	HDL	31729 ± 6095	32250 ± 5148	0.84
	Change in HDL	−1681 ± 8587	411 ± 4127	0.49
	Large HDL	3272 ± 3134	5034 ± 3585	0.24
	Change in L.HDL	221 ± 2341	472 ± 1289	0.76
	Small HDL	28145 ± 4991	27320 ± 6194	0.74
	Change in S.HDL	−1117 (−5582, 1820)	−144 (−2850, 1515)	0.89
	VLDL	2.1 (1.5, 5.3)	3.0 (1.5, 9.8)	0.54
	Change in VLDL	−0.4 (−1.7, 0.5)	−0.1 (−5.1, 1.0)	0.86
IDL-c		50 ± 12	51 ± 13	0.86
Change in IDL-c		−19 (−25, −7)	−13 (−24, −5)	0.73
Lp(a)		6.0 (4.5, 13.0)	11.1 (6.7, 16.6)	0.18
Change in Lp(a)		1.9 ± 5.2	−0.5 ± 4.5	0.28
ApoA1		141 (131, 188)	190 (142, 228)	0.18
Change in ApoA1		−23 (−32, 93)	−20 (−59, 3)	0.73
hsCRP		8.5 ± 2.6	5.7 ± 4.1	0.09
Change in hsCRP		−5.8 ± 2.8	−2.9 ± 3.2	0.04
PCSK9		267 (205, 438)	281 (197, 302)	0.67
Change in PCSK9		4 ± 119	22 ± 74	0.68
Cholesterol efflux capacity		23.7 ± 3.1	21.3 ± 3.0	0.08
Change in cholesterol efflux capacity		−4.1 ± 4.1	−2.0 ± 1.7	0.15
CETP activity		17.1 ± 1.7	16.8 ± 1.8	0.67
Change in CETP activity		0.0 ± 2.1	−0.1 ± 1.6	0.99

lipid content combined with a significant increase in vessel fibrous content after *three months*, and (iv) standard or functional lipid measurements that are currently targeted in drug development did not relate to carotid lipid content at baseline or to its propensity to change with statin treatment.

Plaque lipid depletion in response to LDL-c reduction is widely accepted as a plausible mechanism of benefits using lipid lowering drugs [4,8]. In that model, the reduction in plaque lipid content coupled with the increase in fibrous percentage changes the status of the plaque to one less prone to rupture [4]. Changes in plaque composition have previously been demonstrated in both the ORION and CPC studies [8,9], but only after 12 months of LDL-c lowering treatment and in patients with pre-defined carotid disease. This is important as it reflects both the potential utility and the limited sensitivity of the previous generation of multi-contrast imaging tools used to detect ‘subtle’ changes within short periods of time. Moreover, it restricts the use of

imaging as an approach to monitor response to lipid lowering treatment to those with identified carotid disease. Within three months of statin treatment, only a reduction in carotid atherosclerosis *burden*, as quantified on MRI, has been shown among stable coronary patients [23].

Here we demonstrated *in vivo* that plaque composition changes occur as early as three months. These findings are in-line with previous histological studies [7], but they extend the findings into patients with no pre-defined carotid disease. Notably, the lipid percentage quantified in this study was smaller than previously reported with T2 mapping, presumably reflecting different cohort of ACS as opposed to carotid endarterectomy in previous studies [18,19].

Plaque lipid depletion has been examined invasively, using near infra-red spectroscopy (NIRs), and non-invasively using multi-contrast (MC)-MRI techniques [8,27]. The requirement for a discernible lipid-rich core for lipid quantitation using MC-MRI had restricted the capacity to study changes in plaque composition to a minority of recruited



**Table 3**

Baseline and change in carotid plaque volume and lipid content between patients with LDL-c of more and less 70 mg/dL following statin treatment.

Imaging biomarker	Group 1 (LDL-c < 70 mg/dL)	Group 2 (LDL-c > 70 mg/dL)	p value
Base lipid percentage (%)	9.2 (6.0, 14.3)	10.4 (8.7, 20.2)	0.36
Base plaque volume (mm <sup>3</sup> )	44.8 (34.1, 53.2)	42.7 (41.7, 70.7)	0.51
FU lipid percentage (%)	6.4 (4.9, 9.5)	10.0 (7.5, 12.2)	0.099
FU plaque volume (mm <sup>3</sup> )	43.6 (33.3, 48.8)	45.2 (41.1, 56.3)	0.31
Change lipid percentage (%)	−2.8 (−6.6, −0.3)	−0.9 (−10.2, 1.2)	0.36
Change plaque volume (mm <sup>3</sup> )	−1.7 (−5.9, 0.38)	−2.8 (−14.4, 2.1)	0.84

patients [11]. This was reflected in small proportion of patients, with discernible lipid core (55% of screened patients in ORION and 27% in the CPC studies) [8,9]. This is important as changes in plaques with no identifiable lipid core could not be studied, thus limiting the generalizability of observations of net lipid evacuation in response to LDL-c reduction.

Furthermore, the lack of sensitivity in detecting plaque lipid using previous approaches would have hindered any interpretation of the relationship of blood biomarkers to plaque lipid content [8,28]. Using a highly sensitive T2 mapping MRI technique [18,19], we found a dissociation between plaque lipid content and both standard and functional lipid indices. In other words, state of the art blood biomarkers cannot substitute for direct plaque lipid quantification. This is not surprising since the dynamic range of plaque lipid (potentially accumulating over months to years) was unlikely to be reflected by the relatively narrow range of lipids measures (Supplementary Figure). This dissociation does not overlook the established role of LDL-c as a risk factor in large scale studies, but supports a role for plaque lipid imaging for the characterisation of individual patients as a potential stratification tool.

In this study, we studied changes in vessel lipid content irrespective of plaque type or degree of stenosis. There was almost 30% relative reduction in lipid percentage in subjects presenting with ACS who were commenced on high dose statin therapy. This reduction was mirrored with significant increase in fibrous percentage resembling previously reported *in-vivo* and *ex-vivo* studies [7,8]. Interestingly, changes in carotid lipid percentage in response to LDL-c reduction was not uniform across all patients. Responders tended to have higher lipid% at baseline and were more likely to have larger change in hs-CRP in response to intensive statin treatment. These differences matched previous reports when using invasive techniques in the coronary arteries. In a *post hoc* analysis of the YELLOW trial, the magnitude of reduction in lipid content following 7 weeks of intensive statin treatment quantified using NIRs was proportional to the quantity of lipid amount at baseline [27]. Using intravascular ultrasound combined with virtual histology, the decrease in hs-CRP was greater in patients with reduced necrotic core than those with remaining ones [28]. Our results extend these findings with the benefits of a *non-invasive* imaging technique. Interestingly, none of the studied blood biomarkers demonstrated a significant relationship with changes in lipid percentage, thereby reinforcing the role of plaque imaging in assessing suitability for, and response to, established or new therapies. Moreover, the complex relationship between lipoprotein- and non-lipoprotein related risks, such as diabetes and smoking, undermines the approach of using *risk factors* to guide particular therapies [29]. Instead, we present a tool for *disease characterisation* at individual patient level. This is distinct from the traditional approach of measuring biomarkers, derived from risk factors at population level, which, as we show, do not relate to individual disease or response to therapy.

Direct quantification of plaque lipid, using the T2 mapping technique, may contribute to the identification of patients at greatest risk of atherosclerosis complications, and to the targeting of this patient group with novel lipid lowering drugs [4,30]. The indiscriminate use of these drugs is not practical or affordable and even when successful, the margin of benefits is small, challenging their routine use post ACS

[3,4,31]. Therefore, lipid quantification techniques could be tested as stratification tools to identify patients with persistent high lipid percentage within atherosclerotic plaque after statin treatment and to guide more rational treatment by adding a second lipid modifying drug. In addition, direct quantification of plaque lipid will allow the effects of intensive LDL reduction to be monitored serially with much greater precision than has been possible previously.

The main limitation of our method is the sensitivity to motion artefacts of the DANTE-MESE sequence [18,19]. In this study our overall rejection rate was 13% which is a significant improvement since our initial rejection rate of 35% [19].

This is an observational study using a lipid-lowering intervention that is well-established in clinical practice. This provides a platform to examine changes in plaque composition in using the new T2-mapping technique. It is not a test of the drug *per se* and a randomised placebo-controlled clinical trial would not have been appropriate or ethical in this patient group, where statins form an important part of standard care. The sample size was powered to detect a change in lipid core using a sensitive imaging technique. It is plausible that a much larger study may have drawn out statistically significant relationships between some blood biomarkers and plaque lipid content. However, that is contrary to the intent of this study, which attempts to bring focus and precision to patient characterisation. In addition, the relationship between plaque and blood lipid indices was examined at a single early timepoint, which cannot give definitive information on the full extent or scope for lipid evacuation over a longer time period. Nonetheless, the 3-months interval was selected based on previous evidence of carotid plaque regression within 3 months [23], and to test the utility of T2 mapping as an end point for lipid-lowering interventional trials.

#### 4.1. Conclusions

T2-mapping demonstrated carotid plaque lipid depletion in response to high-intensity statin treatment as early as three months. Existing blood lipid biomarkers did not relate to changes in plaque lipid content with treatment, reinforcing the role of using plaque imaging as a potential tool to identify patients who may be suitable for intensive lipid modification.

#### Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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#### Authors contributions

Conceptualization, investigation, methodology and project administration MA RPC, Data Curation and software MA LB NA FG JTC MDR, Formal analysis MA NA FG RPC, Funding Acquisition MA LB FG MDR,

RPC Writing original draft and preparation MA, RPC, Writing review and editing all authors.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.atherosclerosis.2018.08.033>.

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