Quantitative Magnetic Resonance Imaging Assessment of the Relationships Between Fat Fraction and R2* Inside Carotid Plaques, and Circulating Lipoproteins

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Background: Lipid-rich necrotic core (LRNC) and intraplaque hemorrhage (IPH) are morphological features of high-risk atherosclerotic plaques. However, their relationship to circulating lipoproteins is unclear.

Purpose: To study associations between changes in lipoproteins vs. changes in LRNC (represented by fat fraction [FF]) and IPH (represented by R2*).

Study Type: Prospective.
Subjects: Fifty-two patients with carotid plaques, 33 males (63.5%), mean age 72 (±5).
Field Strength/Sequence: Four-point fast gradient Dixon magnetic resonance imaging (MRI) was used to quantify FF and R2* (to measure IPH) inside plaques and in vessel wall. Turbo-spin echo was used for T1 weighted sequences to guide manual segmentation.
Assessment: Carotid MRI and serum lipid levels were assessed at baseline and at 1-year follow-up. For patients, lipid-lowering therapy was customized to reduce low-density lipoprotein (LDL) levels below 1.8 mmol/L. Segmentation was performed with one set of regions of interest for the plaque and one for the vessel wall at the location of the plaque. Thereby MRI data for FF, R2*, and volumes in plaque- and vessel-wall segmentations could be obtained from baseline and follow-up, as well as changes over the study year.
Statistical Tests: Pearson correlation coefficient for correlations. Paired samples t-test for changes over time. Significance at P < 0.05, 95% confidence interval.

Results: LDL decreased significantly (2.19–1.88 mmol/L, Z = 2.9), without correlation to changes in plaque composition, nor to the significant reduction in vessel-wall volume (−106.3 mm³). Plaque composition remained unchanged, FF +8.5% (P = 0.366) and R2* +3.5% (P = 0.304). Compared to plaque segmentations, R2* was significantly lower in the vessel-wall segmentations both at baseline (−9.3%) and at follow-up (−9.1%).

Data Conclusion: The absence of correlations between changes in lipoproteins and changes in plaque composition indicates more complex relationships between these parameters than previously anticipated. The significant differences in both R2* and volume dynamics comparing plaque segmentations and vessel-wall segmentations suggest differences in their pathobiology of atherosclerosis.

Level of Evidence: 1
Technical Efficacy: Stage 4

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It is well established that statin treatment reduces low-density lipoprotein (LDL) levels and that the reduction of LDL decreases cardiovascular events. Consequently, magnetic resonance imaging (MRI) studies of carotid plaque show a reduction of LRNC in response to statin treatment, indicating a transition toward a more stable plaque phenotype. However, the relationship between lipoprotein cholesterol levels and plaque composition is less clear. Regarding the relationship between LDL and LRNC, diverging outcomes have been presented suggesting both positive associations and a lack thereof. IPH is another well-known marker of high-risk plaques, but dedicated studies exploring the relationship between IPH, statin treatment, and lipid levels have not been performed.

Due to excellent soft-tissue sensitivity, MRI has been widely utilized to characterize atherosclerotic plaques. Typically different combinations of multi-contrast-weighted imaging have been used, in which plaque components are identified by the combination of hypo- or hyperintense signal intensities on T1-, T2*- and protein density-weighted images. The inherent limitation of this multi-contrast approach lies in the method, where plaque features are identified due to calculations of their relative signal intensities. Image analysis thus relies on operator-dependent post-sequence image processing to assess these differences in signal intensity. The resulting data therefore are not a purely quantitative representation of plaque compositional features. Moreover, the MRI sequences used in this approach are not specific for fat and blood. Thus, the accuracy of the measurement of LRNC and IPH is limited. To address these shortcomings a quantitative MRI (qMRI) technique has been developed, that directly measures the physical properties of the relevant plaque components: fat (LRNC) and (heme) iron (IPH). This technique was validated against three-dimensional (3D)-histology showing that fat fraction (FF) and R2* values correlated closely to the volume of LRNC and IPH. Previous studies have examined these relationships while focused primarily on segmentation of the vessel wall in the region of interest, and it is unclear to what extent these measurements reflect changes in the actual plaque.

Thus the aim of this study was to investigate the correlations between changes in lipoproteins and changes in FF and R2*, and to do so both for the plaque and for the circumferential vessel wall.

Materials and Methods

Study Design

This study was designed as a prospective study with repeated measures; it is registered at ClinicalTrials.gov under identifier NCT04835571. It was approved by the Swedish Ethical Review Authority (approval no.: 2016-441-31) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Patients with carotid plaque were selected for the study based on routine duplex ultrasound during the period from January 1, 2012, to February 1, 2017, and 5860 ultrasound investigations were screened. Data collection was between January 10, 2017, and December 6, 2018. Clinical characteristics, laboratory values, clinical events, and medications were recorded for each patient on dedicated case record forms and transferred to a study database.

Patients were selected based on duplex ultrasound criteria established for the European Carotid Surgery Trial. A cut-off was set at a Doppler flow velocity ≥1.3 m/sec, measured at a Doppler angle of 50°–60°, which corresponds to a ≥50% stenosis according to these criteria. Patients up to the age of 80 years were included. Exclusion criteria were: inability to give informed consent, inability to participate in examinations that are necessary for the study (eg, MRI), stroke ≤1 month prior to inclusion, co-morbid conditions (eg, kidney failure with glomerular filtration rate <45 mL/min/1.73 m²), carotid occlusion, patients treated with or planned for carotid endarterectomy or carotid stenting, immunological disorders, neoplastic disease, and patients treated with immunosuppressive/anti-inflammatory agents.

Study Visits

Participants were invited to a study visit at baseline, and to a 1-year follow-up visit. Imaging and blood tests were performed on both occasions. A fasting lipoprotein panel was collected at baseline and follow-up consisting of total cholesterol, LDL, high-density lipoprotein (HDL), non-high-density lipoprotein (NONHDL), Apolipoprotein A1, and Apolipoprotein B1. At the time of the study, the European Guidelines for Cardiovascular Disease Prevention recommended an LDL target of ≤1.8 mmol/L for this patient group. Statins and ezetimibe were prescribed according to clinical routine by the study clinicians (E.G. and E.d.M.) who also kept in touch with the patients over the study year to enhance compliance and oversee additional adjustments if needed.

Data Acquisition

In the same session patients underwent T1-weighted (T1W) imaging used for delineating the carotid vessel wall and the plaque, and four-point Dixon for the quantification of FF and R2*. Data were acquired on a 3-T Ingenia scanner (Philips Healthcare, Best, the Netherlands) using an 8-channel carotid coil (Shanghai Chenguang Medical Technologies, Shanghai, China). MRI was performed with bilateral imaging of the carotid arteries for all patients, but only unilateral data from the side with the most prominent carotid plaque was used in the analysis. Image quality was assessed independently by three team members (E.G., M.Z., P.D.) and imaging data were not used if the quality was considered to be insufficient by at least two of the before-mentioned investigators.

T1W. A turbo-spin echo (TSE) acquisition was performed post administration of gadolinium contrast agent (Gadovist, 0.2 mL/kg/1.0 mL/s), black blood delay time 430 msec. Scan parameters included: echo time (TE) = 9 msec, repetition time (TR) = 1 heartbeat, and TSE factor 6. Slice thickness was 1.75 mm,
and in-plane resolution 0.50 mm × 0.50 mm. The total number of slices was 18 and the axial image stack was vertically centered on the carotid flow divider.

**DIXON.** A Dixon sequence with four echoes was used. A higher number of echoes was avoided based on the fact that the registered R2* values will lose signal magnitude at such a rate that it would be too close to the noise floor after more than four echoes, and therefore an approach using more than four echoes would not increase measurement accuracy. In addition, it would increase scan time. The water-fat shift was maximized at 1.3 pixels, TR = 18 msec, turbo field echo (TFE) = 12, TE = 3.6 msec, and flip angle 10°. Proton density and T1 relaxation differences between water and fat were not considered. Two regional saturation slabs of 80 mm were added inferior and superior to the acquisition volume, with a gap of 40 mm, to suppress signal from inflowing blood. Slice thickness for Dixon images was 0.7 mm, with in-plane resolution 0.60 m × 0.60 m. Acquisition time was 8 minutes.

**Plaque and Vessel-Wall Segmentation**

Manual segmentation of the carotid artery was performed by a reader with 5 years of experience in vascular MRI (E.G.) using ITK-SNAP software and T1W images.21 Segmentations were unilateral and two different segmentations were made for each patient: one segmentation delineating the plaque (defined as a luminal protrusion of the wall ≥1.5 mm in radial thickness) and one segmentation of the whole vessel wall covering the common carotid, carotid bulb, bifurcation, and internal carotid artery. The length of the vessel segmentation was restricted to 30 mm centered on the flow divider.

Sampling of FF and R2*, representing compositional information from the vessel wall was done by first registering the manually generated plaque and vessel segmentations to the Dixon data. Registrations were performed using MATLAB’s (The MathWorks, Natick, MA, USA) imregister function with a One-Plus-One Evolutionary optimizer and the Mattes Mutual Information similarity metric.22,23 The geometric transformation was nonreflective, and allowed to consist of translation, rotation, and scaling. Registrations were visually inspected and manually corrected if necessary. Next, the average FF and R2* value at each voxel was calculated as an average of its immediate neighbors that were within the mask. However, only slice average and whole mask average for FF and R2* were used in the results.

**Outcomes**

Primary outcomes were mean FF and mean R2* for the plaque volume and the vessel-wall volume for each patient at baseline and follow-up, as well as lipoprotein levels at baseline and follow-up. Changes in these values over time were measured, as well as correlations between plaque compositional data and lipoprotein levels.

**Intra-Observer Variability**

To assess intra-observer repeatability scans were analyzed twice, both at baseline and at follow-up for 25% of the patients (10 patients with 2 scans each) regarding both plaque segmentations and whole-vessel segmentations. These measurements were performed by E.G. at two different readings (>12 months apart). Mean FF, R2*, and segmentation volumes were compared.

**Statistical Analysis**

The study was analyzed according to the intention to treat principle to account for non-random attrition of study participants. Consequently, for the analysis of baseline data all patients with sufficient image quality at baseline were included, for the analysis at 12-month follow-up of all patients with sufficient image quality at this time point were analyzed and for paired comparisons between baseline and follow-up only those with sufficient image quality at both time points were taken into account. Hence, to ensure an unbiased analysis, patients with incomplete or missing MRI data were not excluded but contributed to the study with all data that were collected on their behalf.

SPSS Statistics 26 (International Business Machines Corporation, New York, NY, USA) was used for statistical analysis. Continuous variables were summarized as mean ± SD. The strength of the association between lipoproteins, plaque volume, vessel-wall volume, plaque composition, and vessel-wall composition both at baseline and at 1-year follow-up was assessed by calculating the Pearson correlation coefficient. For assessing the distribution of the data, the Shapiro-Wilk test was used. For normally distributed data, paired sample t-test was used for analyzing changes between baseline and follow-up. For data that were not normally distributed, the Wilcoxon signed-rank test was used.

Intra-observer repeatability measurements were calculated using intraclass correlation coefficients (ICC).24 For ICC evaluation, a two-way random effect model was used. In examining absolute agreement, average measures are presented. A P-value of less than 0.05 was considered significant.

**Results**

**Patients**

After applying inclusion and exclusion criteria 88 patients were eligible, and 57 consented to participate. After having given their informed consent, 5 patients ultimately decided not to participate, resulting in 52 patients included in the study. Thus the description of baseline characteristics is based on 52 patients. At the start of the study, a full MRI data set was acquired from 47 subjects. The reason for the lower number of completed scans was that three patients were claustrophobic or uncomfortable in the MRI scanner and could complete the examination neither at baseline nor at follow-up, and for two patients MRI data were lost or of insufficient quality at baseline (but with sufficient MRI data at follow-up). At follow-up, MRI data were available from 41 patients, because two did not wish to return for the follow-up visit and four scans could not be analyzed because of insufficient image quality. Since baseline scans were not available for two patients, ultimately 39 patients had a full MRI dataset from both occasions. In summary, for the comparison between circulating lipid levels and imaging data at baseline 47 patients were available, the same comparison could be made for 41 patients at follow-up and for paired comparisons of baseline and follow-up data 39 patients were available.
Table 1 describes the baseline characteristics of the study participants and their comorbidities. The patients had a mean age of 72 (±5) years, and were predominantly male (63.5%). At study start 44 (84.6%) had a prescribed statin therapy, but 6 patients (11.5%) were on neither statin nor ezetimibe treatment. At follow-up, 49 patients (94.2%) had lipid lowering drugs. During the study, it became apparent that two patients were intolerant to both statins and ezetimibe, therefore lipid lowering medication was discontinued for these two study participants. The types of lipid lowering therapies are shown in Table S1 in the supplemental material. Total cholesterol was significantly reduced from 4.1 mmol/L to 3.8 mmol/L (Z = -2.9). LDL was also significantly reduced, from 2.19 mmol/L to 1.88 mmol/L (Z = -2.9), mean difference -0.28 (–0.44 to -0.12). Apolipoprotein B1 decreased significantly from 0.84 mmol/L to 0.78 mmol/L (Z = -2.9). There was a significant change in the levels of HDL, 1.41–1.35 mmol/L (Z = -2.1), and NONHDL also decreased significantly from 2.71 mmol/L to 2.43 (Z = -2.6). Levels of Apolipoprotein A1 did not show any statistically significant reduction (1.49–1.48 mmol/L, P = 0.62).

### Imaging

Table 2 shows changes in FF, R2*, and volume over the study year both for plaque and for vessel wall. There was a significant decrease in vessel-wall volume from 1496 (±332) mm³ to 1390 (±313) mm³ whereas plaque volume remained unchanged, 608 (±270) mm³ at baseline and 612 (±295) mm³ at follow-up (P = 0.921). As shown in Table 2, no reduction of FF nor R2* was observed, despite the significant reduction in total cholesterol (from 4.1 mmol/L to 3.8 mmol/L, Z = -2.9) and LDL (from 2.19 mmol/L to 1.88 mmol/L, Z = -2.9). Nor were there any significant correlations between changes in total cholesterol or lipoproteins compared to changes in FF, R2*, or volume (Table 3).

Correlations for changes in LDL and plaque composition are shown in Fig. 1a,c, and correlations for changes in LDL and vessel-wall composition are shown in Fig. 1b,d. There were no significant correlations between changes in plaque burden (plaque volume and vessel-wall volume) and changes in FF/R2* over the study year. Pearson correlations for change in plaque volume in relation to change in FF was -0.09 (P = 0.582) for plaque and 0.16 (P = 0.344) for vessel wall, and in relation to change in R2* -0.18 (P = 0.286) for plaque and -0.128 (P = 0.436) for vessel wall. Nor were there significant correlations between changes in FF and changes in R2* for plaque data -0.05 (P = 0.769) and for vessel-wall data -0.04 (P = 0.808) as illustrated in Fig. 1e,f.

Figure 1 also illustrates the highly heterogenous response at an individual patient level, with changes ranging from negligible to a marked decrease or increase in plaque parameters.

The difference between plaque segmentation and whole vessel-wall segmentation is visualized in Figs. 2 and 3. Comparative data for the two types of segmentation at baseline and follow-up and changes in tissue volumes and compositions are presented in Table 4. Comparing all values from plaque segmentation against all values from vessel-wall segmentation, no significant difference in mean FF was observed at baseline (mean difference for plaque segmentations compared to vessel-wall segmentations -0.002%, [P = 0.653]) or at follow-up (–0.004% [P = 0.518]). However, R2* was significantly lower in vessel-wall segmentations, by -9.3% at baseline (mean value 45 s⁻¹ in plaques and 41 s⁻¹ in vessel wall) and by -9.1% (mean value 46 s⁻¹ in plaques and 42 s⁻¹ in vessel wall) at follow-up. Naturally, the volume was significantly larger in vessel-wall segmentations: at baseline (mean value 46 s⁻¹ in plaques and 41 s⁻¹ in vessel wall) and at follow-up mean volume 601 mm³ in plaques and 1374 mm³ in vessel wall.
Intra-Observer Variability

For plaque data, mean FF decreased by −4.7% in repeated segmentation compared to original assessment [ICC 0.959 (0.897–0.984)]. Mean R2* had a difference of −3.0% in repeated segmentation [ICC 0.930 (0.825–0.972)]. Volumes decreased by −7.6% [ICC 0.942 (0.856–0.977)].

For vessel-wall data all ICC were slightly higher. Mean FF was −2.5% in the repeated vessel-wall segmentation compared to the first segmentation [ICC 0.994 (0.982–0.998)]. Mean R2* decreased by −1.5% in repeated vessel-wall segmentation [ICC 0.980 (0.950–0.992)]. Volumes for the vessel-wall segmentations had a variation of only 0.3% [ICC 0.977 (0.942–0.991)].

Discussion

A four-point Dixon 3D gradient-echo sequence was used, with reliable fat and water separation to interrogate the relationship between intraplaque FF and R2* in patients with advanced carotid plaques. A significant reduction in LDL was observed after the optimization of lipid lowering treatment over a time period of 12 months, enabling the study of quantitative changes in plaque composition in response to a reduction in circulating lipoproteins.

Using quantitative MRI there was no correlation between changes in plasma lipid levels vs. changes in plaque burden (volumes of plaque and vessel wall), FF, and R2*. The results of the intra-observer repeatability analysis show that the changes in results between baseline and follow-up cannot be explained by observer-induced variability in the measurements. Although there is unambiguous evidence that treatment that reduces LDL leads to fewer cardiovascular events, the understanding of how the reduction of circulating lipoproteins translates to changes in plaque composition is less clear. Immunohistochemical staining of biopsied carotid endarterectomy samples showed a reduced lipid content in high-grade plaques after 3 months of statin treatment, compared to the group that did not receive statin treatment.27 However, imaging studies have not been uniform in documenting the association between circulating atherogenic lipids and LRNC.28,29 There are studies that show a correlation between cholesterol levels and the presence or absence of LRNC upon MRI. In the MESA study, total cholesterol levels in the middle or high tertile level were associated with the presence of LRNC.30 Likewise in a 4-year follow-up study with serial MRI, there was an association between total plasma cholesterol levels and LRNC as a binary variable.31

On the other hand, in the ARIC study, atherogenic lipoproteins were associated with plaque burden, but not with LRNC.8 Furthermore, in the AIM-HIGH study there were no significant correlations between LDL and LRNC. This lack of correlation persisted after adjusting for age.32 Regarding the effects of lipid lowering treatment several studies have shown a reduction of LRNC in response to statin treatment.5,6 Only one of these studies examined the correlation between change in LRNC and LDL levels and found no statistically significant correlation between the two.5

The previously documented absence of a correlation between LDL levels and LRNC is consistent with the quantitative results from this study. A non-linear relationship between LDL and LRNC agrees with the complex interplay of factors that balance plaque progression vs. regression. The influx of LDL and small lipoproteins is a mechanism that promotes plaque progression.5,6 Others are systemic inflammation, co-morbidities like diabetes mellitus and hypertension, local shear stress in the artery, genetic determinants of arterial wall biology, and lifestyle parameters like smoking.33,34 Plaque regression is possible in an environment that favors a Th2 cell response and M2 macrophage response, upregulating efferocytosis, efflux of lipids from the plaque,

| Segmentation | Mean Value at Baseline | Mean Value at Follow-Up | Difference (%) | P      |
|--------------|------------------------|-------------------------|----------------|--------|
| Fat fraction (%) | Plaque | 0.142 ± 0.051 | 0.154 ± 0.066 | +8.5 | 0.366 |
| Vessel wall | 0.138 ± 0.053 | 0.149 ± 0.064 | +8.0 | 0.386 |
| R2* (s⁻¹) | Plaque | 45.1 ± 11.1 | 46.7 ± 12.2 | +3.5 | 0.304 |
| Vessel wall | 41.0 ± 8.1 | 42.5 ± 8.9 | +3.6 | 0.238 |
| Volume (mm³) | Plaque | 608 ± 270 | 612 ± 295 | +0.5 | 0.921 |
| Vessel wall | 1496 ± 332 | 1390 ± 313 | −7.1 | <0.05 |

Changes in plaque composition and volume between baseline and follow-up, using paired samples. Data were retrieved from two modes of segmentation: plaque segmentation and vessel-wall segmentation in the region of interest. N = 39. There was a significant decrease in vessel volume, whereas there were no significant changes in plaque composition over the study year.
### TABLE 3. Correlations Between Plasma Lipids, Plaque Composition, and Volume for Plaque Segmentations as Well as Vessel-Wall Segmentations

| Visit | Total cholesterol | HDL | LDL | NONHDL | Apolipoprotein A1 | Apolipoprotein B1 | Total cholesterol | HDL | LDL | NONHDL | Apolipoprotein A1 | Apolipoprotein B1 |
|-------|------------------|-----|-----|--------|------------------|------------------|------------------|-----|-----|--------|------------------|------------------|
| Baseline | FF Plaque | P | FF Vessel Wall | P | R2* Plaque | P | R2* Vessel Wall | P | Volume Plaque | P | Volume Vessel Wall | P |
| | | | | | | | | | | | | | |
| Baseline | 0.017 | 0.908 | 0.105 | 0.479 | 0.028 | 0.850 | 0.194 | 0.187 | 0.170 | 0.254 | 0.131 | 0.375 |
| HDL | 0.183 | 0.217 | 0.197 | 0.180 | 0.075 | 0.615 | 0.062 | 0.676 | 0.069 | 0.647 | 0.094 | 0.526 |
| LDL | 0.018 | 0.902 | 0.077 | 0.605 | 0.001 | 0.992 | 0.020 | 0.153 | 0.303 | 0.166 | 0.258 |
| NONHDL | 0.055 | 0.714 | 0.023 | 0.876 | 0.000 | 1.000 | 0.023 | 0.104 | 0.188 | 0.205 | 0.200 | 0.173 |
| Apolipoprotein A1 | 0.010 | 0.946 | 0.062 | 0.674 | 0.011 | 0.944 | 0.022 | 0.121 | 0.197 | 0.186 | 0.136 | 0.358 |
| Apolipoprotein B1 | 0.016 | 0.923 | 0.022 | 0.893 | 0.066 | 0.682 | 0.039 | 0.813 | 0.270 | 0.088 | 0.082 | 0.613 |
| Follow-up | Total cholesterol | 0.082 | 0.611 | 0.008 | 0.959 | 0.215 | 0.178 | 0.033 | 0.167 | 0.303 | 0.136 | 0.464 |
| HDL | 0.019 | 0.907 | 0.010 | 0.952 | 0.013 | 0.937 | 0.019 | 0.908 | 0.274 | 0.083 | 0.031 | 0.847 |
| LDL | 0.048 | 0.763 | 0.005 | 0.973 | 0.030 | 0.853 | 0.015 | 0.926 | 0.214 | 0.179 | 0.033 | 0.838 |
| NONHDL | 0.047 | 0.771 | 0.097 | 0.551 | 0.177 | 0.269 | 0.124 | 0.446 | 0.086 | 0.595 | 0.218 | 0.177 |
| Apolipoprotein A1 | 0.031 | 0.846 | 0.002 | 0.988 | 0.086 | 0.591 | 0.025 | 0.878 | 0.206 | 0.196 | 0.011 | 0.946 |
| Apolipoprotein B1 | 0.058 | 0.727 | 0.007 | 0.948 | 0.059 | 0.720 | 0.045 | 0.784 | 0.208 | 0.205 |

Pearson correlations are shown for lipoproteins in relation to plaque composition and volume. Plaque compositional data were calculated for plaque segmentations and for segmentations of the vessel wall in the region of interest. For baseline results data from 47 patients with a complete MRI dataset was used, for follow-up 41 patients, and for the difference between baseline and follow-up data from 39 patients that had a full MRI dataset from both occasions was available. There were no significant correlations at baseline (top rows) and at follow-up (middle rows) and nor were there any significant correlations in differences from baseline to follow-up (lower rows).

FF = fat fraction; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NONHDL = non-high-density lipoprotein.
and the resolution of inflammation.\textsuperscript{35,36} In summary, the environment that regulates plaque homeostasis is constituted not only of atherogenic lipoproteins, but a wide array of pro- and anti-atherogenic factors. Therefore, the measurement of changes in LDL, or any other single risk factor, may not sufficiently reflect the balance between pro- and anti-atherogenic stimuli.

No correlation between changes in plaque burden (vessel wall and plaque) and FF and R2* was found in the analysis of the relationship between atherosclerotic burden and plaque composition. Nor were there any correlations between FF and R2*. However, when compared to previously established data on the natural course of carotid plaque development, a slower plaque progression was observed in this population with an increase in volume of 3.3 mm$^3$, and a significant reduction of plaque burden in vessel-wall segmentations. Previously natural progression has been reported at rates of at least +20 mm$^3$/year.\textsuperscript{37} Thus, data from this study indicate a slowing of disease progression in response to statin treatment, which might be explained by the known pleiotropic effect of statins, rendering stabilizing changes within the plaque independent of levels of circulating lipids.\textsuperscript{13} Importantly, this segmentation approach shows that atherosclerotic vessel segments without plaque behave differently compared to plaque per se.

In this study, measurements of compositional data in the volume of the plaque but also the volume of the entire vessel wall along the plaque length have yielded new insights in the distribution of FF and R2*. This study is the first to quantitatively compare the tissue-specific composition of the plaque against the vessel wall. FF was equally high in the most vulnerable area, i.e., the plaque, and the vessel wall. However, the same patients that have equal levels of FF concurrently showed significantly lower levels of R2* in vessel-wall segmentations compared to plaque segmentations, both at baseline and follow-up. It is well established that atherosclerotic disease is not restricted to the focal protrusion from the vessel wall, i.e., atherosclerotic plaques.\textsuperscript{38} Differences in pathobiology between plaque and adjacent atherosclerotic vessel changes have been demonstrated previously. For example, engagement of plaque-free arterial segments in patients with known atherosclerotic disease is presented in an MRI/PET study of 755 individuals from the Progression of Early Subclinical Atherosclerosis (PESA) cohort. Inflammatory activity was predominantly located in plaque-free areas with 61.5% of the vessel wall showing increased inflammation vs. 38.5% within detectable plaques, thus, providing evidence of generalized inflammation in atherosclerotic wall segments.\textsuperscript{19}

**Limitations**

The small study size was a major limitation as well as the fact that it was performed as a single-center study, using a single MRI scanner and single field strength. It cannot be dismissed that a larger sample size might have yielded significant

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\caption{Correlations between changes in low-density lipoprotein levels and plaque composition for plaque segmentations as well as vessel-wall segmentations. Relative change at follow-up is shown for each study participant with respect to low density lipoprotein levels, fat fraction and R2* values. The data from plaque segmentations are shown in the upper panels (a, c, e), the data from vessel-wall segmentations are shown in the lower panels (b, d, f). Pearson correlation showed no significant correlations between any of the changes. FF = fat fraction; LDL = low-density lipoprotein.}
\end{figure}
correlations between circulating lipoproteins, plaque volume, and plaque composition. However, smaller studies using imaging methods that were not purely quantitative have shown a reduction of LRNC upon statin treatment.\textsuperscript{4–7} Moreover, the follow-up times in two of these studies were less than a year.\textsuperscript{5,6} The well-validated quantitative imaging methods applied in the current study were unable to corroborate these findings.

Another limitation was the lack of a control group, which was excluded for ethical reasons. Earlier results show evidence of a high cardiovascular event rate in patients with high-grade carotid plaques, while secondary prevention for

FIGURE 2: Illustrations of plaque segmentations and vessel-wall segmentations. Magnetic resonance imaging (MRI) imaging shown in axial (left), sagittal (mid), and coronal (right) views, demonstrating T1W and Dixon sequences from one of the patients. The two methods of segmentation of the carotid artery are shown in two panels: (a) plaque segmentation in red (upper panel), (b) vessel-wall segmentation in blue (lower panel).
these patients is inadequate. To withhold optimized treatment from study participants would have caused a higher risk for future catastrophic events on an individual basis. Furthermore, to ensure repeatability, an inter-observer analysis would have been helpful. Such an analysis was not performed because of the lack of another team member with experience in manual plaque segmentation. Another limitation is the fact that follow-up was not extended beyond 1 year. A longer

FIGURE 3: Illustrations of plaque segmentations and vessel-wall segmentations. Samples of segmented volumes, visualizing the two separate strategies for plaque segmentation and vessel-wall segmentation with examples from the common carotid artery, the bifurcation and the internal carotid artery. The images are black-blood T1W with manual segmentations shown in red. Plaque segmentation (a) and vessel-wall segmentation (b) for one segment of the common carotid artery. Plaque segmentation (c) and vessel-wall segmentation (d) for the bifurcation. Plaque segmentation (e) and vessel-wall segmentation (f) for the internal carotid artery.
follow-up might have shown reductions in FF and R2* at group level. Previous MRI studies indeed indicate that LRNC is reduced within 3 months after initiation of statin treatment, but several other studies show significant differences only after 12 months or more of lipid lowering therapy. In this study, a longer follow-up might have shown statistically significant reductions in FF and R2*. Similarly, it is possible that the same treatment would have resulted in significant plaque changes in patients with a lesser degree of stenosis. Since the current study only included patients with carotid stenosis ≥50%, this question needs to be addressed in future studies. Finally, the LDL target used at the time of the study was higher than currently recommended LDL levels. Guidelines for cardiovascular prevention regard the time of the study was higher than currently recommended LDL levels. Guidelines for cardiovascular prevention regarded patients with very high cardiovascular risk recommended an LDL target of ≤1.8 mmol/L at the time, which more recently has been lowered to ≤1.4 mmol/L. It is possible that more aggressive lipid lowering treatment would have resulted in more pronounced changes in plaque composition and volume.

**Conclusion**

This study showed that the relationships between circulating atherogenic lipoproteins and atherosclerotic plaque biology are complex and non-linear. The significant changes in circulating lipoproteins were not correlated with the significant reduction in atherosclerotic burden that was observed in vessel-wall segmentations, nor were they correlated to changes in plaque composition. Moreover, the reduction in atherosclerotic burden showed no correlation to changes in plaque composition. Finally, the significant reduction in vessel-wall volume and the significantly higher R2* values in plaque segmentations vs. vessel-wall segmentations may indicate differences in the pathobiology of atherosclerosis between plaque and vessel wall. Thus, in future studies of the relationship between circulating biomarkers and imaging biomarkers of atherosclerosis, these two regions may need to be evaluated separately.

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| Visit               | Relative Change (%) | Mean Diff | Std Err Mean | t     | N   | P     |
|---------------------|---------------------|-----------|--------------|-------|-----|-------|
| Baseline            | Fat fraction (%)    | −1.4      | −0.002       | 0.004 | 47  | 0.653 |
|                     | R2* (s⁻¹)           | −9.3      | −3.81        | 1.15  | 47  | <0.05 |
|                     | Volume (mm³)        | +58.5     | 854.6        | 47.2  | 47  | <0.05 |
| Follow-up           | Fat fraction (%)    | −2.9      | −0.004       | 0.01  | 41  | 0.518 |
|                     | R2* (s⁻¹)           | −9.1      | −3.86        | 1.31  | 41  | <0.05 |
|                     | Volume (mm³)        | +56.1     | 770.2        | 43.4  | 41  | <0.05 |

Comparison of plaque data and vessel-wall data using paired samples t-test based on the 47 patients at baseline, and the 41 patients at follow-up that had a complete MRI dataset. Plaque data are subtracted from whole-vessel data, showing significantly higher levels of R2* in the plaque segmentation compared to the vessel-wall segmentation both at baseline and follow-up. As is to be expected, the volume is significantly smaller for the plaque compared to the whole vessel segmentation. For FF and R2* mean values are used, for volume the total segmented quantity is presented. 95% confidence interval of the difference is used for lower and upper limits. FF = fat fraction; N = number of patients.
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