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Change in Carotid Plaque Components

A 4-Year Follow-Up Study With Serial MR Imaging

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ABSTRACT

OBJECTIVES The goal of this study was to determine how carotid plaque components (e.g., intraplaque hemorrhage [IPH], calcification, lipid core) change over time and which cardiovascular risk factors are associated with the development of each component.

BACKGROUND Carotid atherosclerotic plaque components are important markers of plaque vulnerability. How these components change and which factors lead to the development and changes in the components remain unclear.

METHODS A total of 198 participants (mean age 67.5 ± 10.6 years) from the population-based Rotterdam Study, all with carotid wall thickening on ultrasound, underwent 2 magnetic resonance imaging scans for carotid plaque characterization (mean interscan interval 4.1 ± 0.2 years). Presence of IPH, calcification, and lipid-rich necrotic core was assessed on both sides on the baseline and follow-up scans. The association between cardiovascular risk factors and incident carotid plaque components was assessed.

RESULTS In the 396 arteries, all plaque components significantly changed over time. Incidence of IPH, calcification, and lipid core was, respectively, 18.5%, 59.2%, and 39.6%. The factor most strongly associated with the incidence of IPH was use of antihypertensive drugs (multivariate adjusted odds ratio [OR]: 3.87; 95% confidence interval [CI]: 1.90 to 7.90) and severe hypertension (multivariate adjusted OR: 4.70; 95% CI: 1.50 to 14.80). The incidence of calcification was associated with hypertension (OR: 2.20; 95% CI: 1.07 to 4.40). Higher cholesterol levels were associated with incidence of lipid cores (multivariate adjusted OR per unit increase in cholesterol: 1.40; 95% CI: 1.10 to 1.70).

CONCLUSIONS In these community-dwelling subjects, characteristics of plaque composition changed dramatically within a few years, and cardiovascular risk factors played a major role in these changes. Hypertension and its treatment and serum cholesterol levels were the main risk factors for the development of atherosclerotic plaque components over time. (J Am Coll Cardiol Img 2018;11:184–92) © 2018 by the American College of Cardiology Foundation.

Atherosclerosis is a gradually progressive process that leads to cardiovascular diseases (CVD), currently the number one cause of mortality and morbidity (1). In the pathophysiological mechanisms leading from atherosclerosis to CVD, it is increasingly understood that factors other than stenosis and plaque volume are clinically important; for example, lipid-rich necrotic core (LRNC), intraplaque hemorrhage (IPH), inflammation, and thin fibrous caps are strong predictors of plaque vulnerability to rupture (1–4). In the case of plaques located at the carotid arteries, increased vulnerability might lead to thromboembolism and cerebral ischemia (5). The factors that lead to the development of vulnerable plaques are not yet well determined and may be of great relevance to improve the prevention, treatment, and prediction of CVD.

To better understand plaque progression from stable lesions to rupture-prone plaques, serial in vivo imaging of the atherosclerotic plaques is needed.

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Knowledge derived from this study could be useful for risk prediction of cardiovascular events, as well as for monitoring the efficacy of treatment (1). High-resolution magnetic resonance imaging (MRI) recently has enabled accurate noninvasive in vivo identification of plaque components and the remodeling process of carotid atherosclerotic plaques (3,6–11).

A few cross-sectional studies have shown the relation between cardiovascular risk factors and the presence of different plaque components. However, changes in presence of IPH, calcification, and LRNC over time, as well as its determinants, have not been well studied. Although it has been recently shown that plaque growth is influenced by plaque composition and drug intake, the role that cardiovascular risk factors might play in these changes is still unclear (6,12).

The goal of the present study was to investigate the incidence of plaque components over time and to evaluate the factors associated with the development of each plaque component. We used serial carotid MRI in 198 community-dwelling subjects with subclinical carotid atherosclerosis.

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PATIENTS AND METHODS

STUDY POPULATION. This study is embedded within the population-based Rotterdam Study, a prospective cohort study investigating determinants of various chronic diseases that has been ongoing since 1990 in the city of Rotterdam, the Netherlands, among community-dwelling subjects ≥45 years of age. From October 2007 onward, carotid MRI scanning was performed in all Rotterdam Study subjects with a carotid wall thickness ≥2.5 mm on either 1 or both sides on carotid ultrasound. Excluding study participants with contraindications for MRI, previous carotid endarterectomy, or poor image quality on MRI resulted in 1,739 MRI examinations. Approximately 4 years after the first scans, the first 331 subjects with a baseline MRI were invited to undergo a second MRI scan. From all participants invited to undergo the follow-up scan (N = 331), 12.4% (n = 41) had died; from the remaining participants (n = 290), 202 responded (69.6%). Four participants were excluded because of bad imaging quality. Subjects with carotid wall thickness on ultrasound underwent 2 MRI scans with a 4-year interval (n = 198).

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants. The procedures were in accordance with institutional guidelines and the Declaration of Helsinki (13).

RISK FACTOR ASSESSMENT. All risk factors were measured before the baseline MRI scans, and home interviews were performed to collect information on medical history and smoking behavior. Smoking was stratified as current, past, or never. Medication use data were obtained either by automated linkage to pharmacies with computerized records or by self-report. Antihypertensive drug use was assessed by self-report and included alpha-blockers, diuretic agents, beta-blockers, calcium-channel blockers, and angiotensin-converting enzyme inhibitors. Study center visits were performed to measure blood pressure. Hypertension and its levels of severity were defined according to the European Society of Cardiology Hypertension Guidelines, which defines mild hypertension as systolic blood pressure (SBP) 140 to 159 mm Hg and/or diastolic blood pressure (DBP) 90 to 99 mm Hg, moderate hypertension as SBP 160 to 179 mm Hg and/or DBP 100 to 109 mm Hg, and severe hypertension as SBP ≥180 mm Hg and/or DBP ≥110 mm Hg (14). We have adjusted the blood pressure parameters for the patients undergoing antihypertensive treatment, adding 15 and 10 mm Hg to SBP and DBP, respectively. This approach has been shown to be more effective than adjusting for antihypertensive drug use, excluding these individuals, or ignoring this fact (15).

Levels of total cholesterol and high-density lipoprotein cholesterol were measured by using standard laboratory techniques (16). Diabetes mellitus was defined according to the 1997 American Diabetes Association criteria as the use of antidiabetic medication and/or a nonfasting serum glucose level ≥11.1 mmol/l (200 mg/dl) and/or fasting serum glucose levels ≥7 mmol/l (≥126 mg/dl). Height and weight were measured, and the body mass index was calculated.

MRI ACQUISITION. All MRI scans were obtained with a 1.5-T scanner. A standard scanning protocol was used with a scanning time of approximately 30 min. Participants were stabilized in a custom-designed head holder. Thereafter, high-resolution MRI sequences were planned to image the carotid bifurcations on both sides (17). There were 4 sequences in the axial plane: a proton density weighted (PDw)-fast spin echo (FSE)-black blood (BB) sequence; a PDw-FSE-BB with an increased in-plane

ABBREVIATIONS AND ACRONYMS

3D = three-dimensional
BB = black blood
CI = confidence interval
CT = computerized tomography
CVD = cardiovascular diseases
DBP = diastolic blood pressure
EPI = echo planar imaging
FSE = fast spin echo
IPH = intraplaque hemorrhage
MRA = magnetic resonance angiography
MRI = magnetic resonance imaging
LRNC = lipid-rich necrotic core
OR = odds ratio
PC = phased contrast
PDw = proton density weighted
SBP = systolic blood pressure
T1w = T1-weighted
T2w = T2-weighted

resolution; a PDw-echo planar imaging (EPI) sequence; and a T2-weighted (T2w)-EPI sequence. There were 2 three-dimensional (3D) sequences: a 3D-T1-weighted (T1w)-gradient echo sequence; and a 3D-phased contrast-magnetic resonance angiography (3D-PC-MRA). Two-dimensional time-of-flight MRA was performed to cover the carotid bifurcation at both sides, ranging from 15 mm caudal to 30 mm cranial from the bifurcation. The same 1.5-T scanner and protocol were used for the baseline and follow-up scans (GE Healthcare, Milwaukee, Wisconsin) with a bilateral phased-array surface coil (Machnet, Eelde, the Netherlands). No scanner hardware or software changes were made during the study period.

IMAGE ANALYSIS. The quality of all sequences in each MRI scan was rated on a 5-point scale (1 = worst; 5 = best). Scans were included in the analyses if the image quality was ≥3 on all sequences. We assessed plaque characteristics in all plaques with a carotid wall thickness ≥2.0 mm on MRI (n = 352 on baseline scans and n = 378 on follow-up scans). Plaques were reviewed for prevalence of 3 different plaque components: calcification, IPH, and LRNC. IPH was defined as a hyperintense region in the atherosclerotic plaque on the 3D-T1w-gradient echo sequence. Calcification was defined as a hypointense region on all sequences but mainly on the 3D-PC-MRA sequence. LRNC was defined as a region not classified as IPH or calcification on PDw-FSE, PDw-EPI, and T2w-EPI images, and with a relative signal-intensity drop in the plaque on the T2w-EPI sequence (18,19). All sequences were used in parallel to characterize the plaque composition.

The MRI scans were analyzed by 1 of 3 independent trained observers blinded to any subject characteristics regarding the presence of plaque components (IPH, calcification, and LRNC). Two observers analyzed the baseline scans, and 2 observers analyzed the follow-up scans, 1 of whom was the same in both time points (but with a considerable time lag, and all raters were blinded to the initial rating). Due to the difference in time periods in which baseline and follow-up scans were read, the observers were not blinded for the time point of scans. Interobserver agreement was measured based on the rating of 60 carotid arteries from 30 subjects, randomly selected from the database, by 2 reviewers blinded to any subject characteristics. Cohen's kappa statistics revealed good agreement for IPH (0.77; 95% confidence interval [CI]: 0.67 to 0.87) and calcification (0.85; 95% CI: 0.78 to 0.92) and moderate agreement for LRNC (0.44; 95% CI: 0.33 to 0.55). Intra-observer variability was measured in 40 participants who underwent a second MRI scan (average time between scans 15 ± 9 days). These scans were examined by the same physician who performed the initial readings, blinded to the results from the initial evaluation. The intra-observer agreement was good for all measurements. The kappa values were 0.95 (95% CI: 0.88 to 0.99) for the presence of IPH, 0.85 (95% CI: 0.74 to 0.96) for LRNC, and 0.91 (95% CI: 0.82 to 0.99) for calcification.

Carotid bifurcation was visualized in the field-of-view, and the full extent of plaque was visualized on all arteries with plaques. Change of plaque components was categorized as incidence (component not present on baseline but present on follow-up scan), persistence (component present on both baseline and follow-up scans), regression (component present on baseline but not on follow-up scan), or absence (component not present either on baseline or on follow-up scan) (**Figures 1 and 2**).

Baseline wall volume measurement was performed by an automated tool, which quantifies carotid vessel wall volume and is based on a deformable model fitting with a learning-based correction of systematic errors. By selecting 2 initialization points, the tool automatically determines the vessel wall volume in a region around the bifurcation. This automatic measurement performs comparably to manual measurements (5).

STATISTICAL ANALYSES. Statistical analyses are described in the Online Appendix.

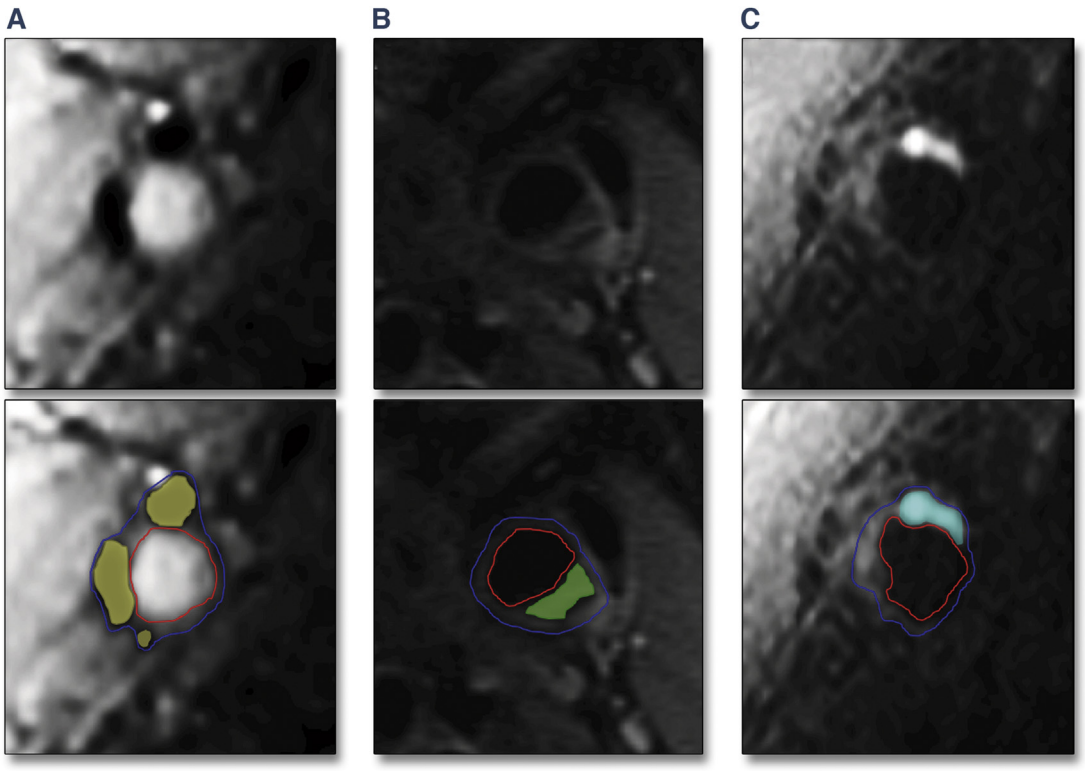
RESULTS

A total of 198 participants (396 carotid arteries) were included. The average age of the participants was 67.50 ± 10.63 years, and 57.1% were men (**Table 1**). Follow-up time was 4.1 ± 0.2 years. The p values for the differences in baseline characteristics between men and women are described in **Table 1**. We found 352 arteries with wall thickness measuring ≥2 mm at baseline, and this number increased to 378 on follow-up scans. There was a significant change in the proportion of presence of all 3 plaque components between the baseline and follow-up scans (**Table 2, Figure 3**). Differences in plaque composition between men and women both on baseline and follow-up scans were not statistically significant.

Table 3 and Figure 3 illustrate changes between baseline and follow-up plaque components in the 396 carotid arteries. Incidence of IPH, calcification, and LRNC were 18.5%, 59.2%, and 39.6%, respectively. The percentages of regression are also shown in **Table 3**.

Table 4 shows the associations between cardiovascular risk factors and the incidence of plaque components. The risk factors most strongly and

FIGURE 1 **Plaque Components on MRI Scans**



Carotid artery magnetic resonance images (1.5-T) of different atherosclerotic plaque components. The **top row** shows the original images; the **bottom row** shows, for visualization purposes, the various components drawn in the original images. **(A)** Calcified plaque: Hypointense region in the plaque in all sequences. Shown is a 3D-PC-MRA. **(B)** LRNC: Hypointense to isointense signal on PDw-EPI sequence. **(C)** IPH: Hyperintense signal on 3D-T1w-GRE sequence. **Red line** = lumen, wall boundary. **Blue line** = wall, perivascular tissue boundary. **Yellow** = calcification. **Green** = LRNC. **Blue** = IPH. GRE = gradient recalled echo; IPH = intraplaque hemorrhage; LRNC = lipid-rich necrotic core; MRA = magnetic resonance angiography; MRI = magnetic resonance imaging; PC = phased contrast; PDw = proton density weighted; T1w = T1-weighted; 3D = 3-dimensional.

independently related to IPH incidence were use of antihypertensive drugs (multivariable adjusted OR: 3.9; 95% CI: 1.89 to 7.93) and severe hypertension (multivariable adjusted OR: 4.72; 95% CI: 1.50 to 14.80). Age was also found to be an independent risk factor for incidence of hemorrhage (multivariable adjusted OR per year increase: 1.07; 95% CI: 1.02 to 1.11). Hypertension exhibited an association with calcification incidence (OR: 2.16; 95% CI: 1.07 to 4.37). This association remained after multivariate adjustments, although not statistically significant. Cholesterol levels were associated with newly detectable LRNC both in models 1 and 2 (multivariate adjusted OR per unit increase in cholesterol: 1.37; 95% CI: 1.08 to 1.74). Female sex was an independent protective factor for incidence of LRNC (OR: 0.48; 95% CI: 0.27 to 0.85).

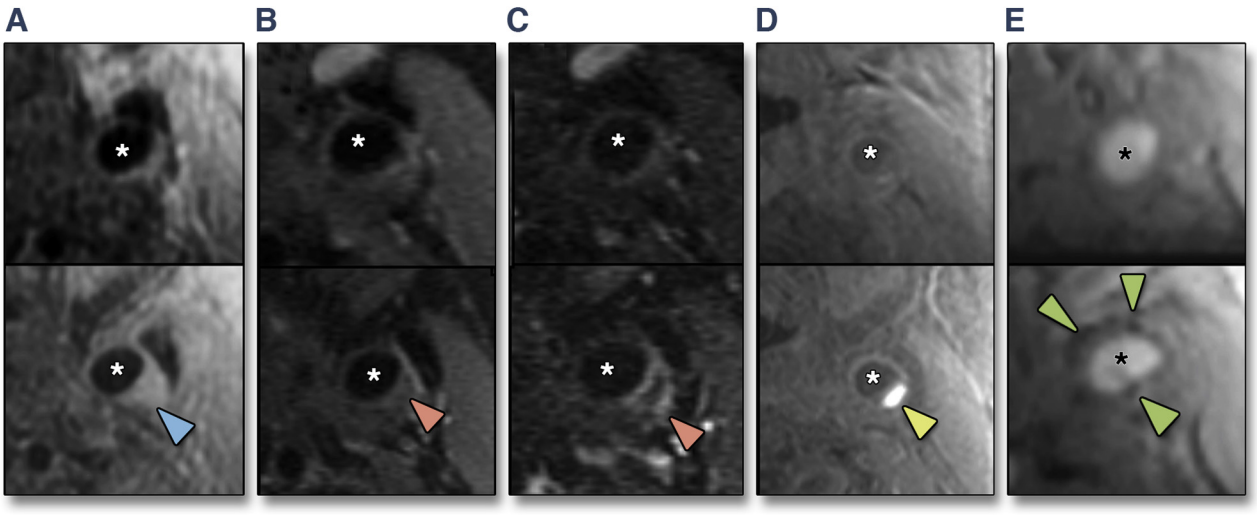
We performed a secondary analysis to relate cardiovascular risk factors with the combined outcome incidence and/or persistence of the plaque components (Online Table 1). In this secondary analysis, findings similar to the analyses with incident components were found.

Analyses stratified according to sex are shown in Online Table 2. The main results are in accordance with the overall analyses, although with less statistical significance (probably owing to the smaller sample size within each stratum).

DISCUSSION

In approximately 200 community-dwelling subjects, we studied changes in plaque composition over time by using 2 serial carotid MRI scans. Overall, there was a dramatic change in components with incidence appearance of 18.5%, 59.2%, and 39.6% for IPH, calcification, and LRNC, respectively. Our findings indicate that traditional cardiovascular risk factors are independent determinants of change in carotid

FIGURE 2 Incidence of Carotid Plaque Components



Plaque in the left carotid artery at the same level in both baseline and follow-up scan. **A to E** represent an axial cross-section of the common carotid artery (CCA) in the same subject. The upper row represents baseline scans; the bottom row represents follow-up scans. *Indicates the lumen of the CCA. **(A)** PDw-FSE-BB image with an increased in-plane resolution shows a plaque as a thickened arterial wall **(blue arrowhead)**. Baseline and follow up PDw-EPI images **(B)** and EPI-T2w **(C)** sequence show LRNC as hypointense to isointense signal with a relative signal intensity drop **(pink arrowhead)**. **(D)** IPH shown as a hyperintense signal on 3D-T1w-GRE sequence on follow-up scan **(yellow arrowhead)**, not seen on baseline scan. **(E)** Calcification shown as hypointense signal on 3D-PC-MRA sequence on follow-up scan **(green arrowheads)**, not seen on baseline scan. EPI = echo planar imaging; other abbreviations as in Figure 1.

plaque composition over time. Older age, antihypertensive drug use, and severe hypertension were independently associated with incidence of IPH. Hypertension was associated with calcification incidence and/or persistence, and higher cholesterol levels were associated with incident LRNC.

TABLE 1 Baseline Population Characteristics (N = 198)

	Men (n = 113)	Women (n = 85)	p Value	All (N = 198)
Age, yrs	67.22 ± 10.80	67.88 ± 10.45	0.668	67.51 ± 10.63
Systolic blood pressure, mm Hg	141.55 ± 18.56	140.82 ± 23.33	0.811	141.24 ± 20.69
Diastolic blood pressure, mm Hg	80.94 ± 10.01	79.18 ± 11.49	0.247	80.18 ± 10.68
Use of antihypertensive drugs	44.00 (38.94)	35.00 (41.18)	0.750	79.00 (39.90)
Hypertension	82.00 (72.57)	55.00 (64.71)	0.236	137.00 (69.19)
Mild	50.00 (44.25)	29.00 (34.12)	0.241	79.00 (39.90)
Moderate	26.00 (23.01)	17.00 (20.00)	0.241	43.00 (21.72)
Severe	6.00 (5.31)	9.00 (10.59)	0.241	15.00 (7.58)
Total cholesterol, mmol/l	5.11 ± 1.08	5.89 ± 1.07	0.000	5.45 ± 1.14
Statins use	40.00 (33.54)	23.00 (27.06)	0.212	63.00 (31.82)
HDL cholesterol, mmol/l	1.21 ± 0.33	1.54 ± 0.41	0.000	1.35 ± 0.40
Diabetes mellitus	21.00 (18.58)	12.00 (14.12)	0.404	33.00 (16.67)
Body mass index, kg/m^2	27.60 ± 3.22	27.23 ± 4.19	0.504	27.44 ± 3.66
Current smoking	21.00 (18.58)	13.00 (15.29)	0.000	34.00 (17.17)
Past smoking	77.00 (68.14)	39.00 (45.88)	0.000	116.00 (58.59)
Never smoked	15.00 (13.27)	33.00 (38.82)	0.000	48.00 (24.24)
Vessel wall volume, mm^3	1.07 ± 0.52	1.00 ± 1.34	0.472	1.04 ± 0.96
Follow-up time, yrs	4.15 ± 0.18	4.14 ± 0.14	0.450	4.15 ± 0.16

Values are mean ± SD or n (%).
HDL = high-density lipoprotein.

STUDY LIMITATIONS. To our knowledge, this is the first in vivo serial MRI study that qualifies changes in 3 plaque components (IPH, calcification, and LRNC) and investigates its determinants in a community-based cohort. High-resolution MRI scans have been shown to be a reliable, noninvasive, in vivo method of detecting atherosclerotic plaque components (8,9,11,19,20). Former studies have focused mainly on 1 or 2 plaque components, or simply acquired cross-sectional MRI scans, with no evaluation of change in plaque composition over time. Another strength of our study is that we focused on community-dwelling subjects with subclinical atherosclerosis. This approach has the advantage of minimizing the potential of selection bias, as well as the chance that the associations found were influenced by medical interventions or lifestyle changes.

Our study has some limitations. The sample size limits the capacity to detect associations between the different factors evaluated and to conduct subgroup analyses. Because we were analyzing an elderly population, with prevalent cardiovascular risk factors and over a long period, there was a high chance of

loss of follow-up because of death and comorbidity, which could be related to the outcomes being studied. In addition, the study population was at higher risk of developing new plaque components because the subjects were preselected based on ultrasound screening for carotid plaque presence. Because we were only considering participants who were alive at the follow-up stage, the opportunity was possibly lost to study the most vulnerable plaques, which might have led to cardiovascular events.

On performing the adjustment for SBP and DBP parameters in patients undergoing antihypertensive treatment, we may be oversimplifying the effect of these drugs because subjects take different doses of different drug classes. Although the interobserver agreement was excellent for IPH and calcification (0.77 and 0.85, respectively), it was fair to good (0.44) for LRNC. However, the intra-observer agreement was almost perfect for all 3 plaque components. In addition, there is a chance that the incident components were already present at baseline but too small to be detected by MRI scanning; thus, they have been classified as incident while in fact they were persistent. Therefore, the number of incidence and regression of the components may, in reality, be lower than we have presented. Furthermore, new foci of plaque components were not classified as incidence, if the component was already present at baseline, but as persistence. To decrease this potential misclassification, a secondary analysis was performed considering incidence and persistence as a combined outcome (Online Table 1). This analysis rendered similar results. Because we are investigating qualitative change in plaque components, and not quantitative change in plaque or component volume, we may be missing more detailed information on change of plaque components volume over time. In addition, because the number of cases showing regression of plaque components was limited, the statistical power was compromised, which did not allow us to analyze this outcome.

CHANGE IN PLAQUE COMPOSITION. Our results show that all 3 plaque components substantially changed over time, not only increasing in presence but also regressing to absence in a limited number of cases (**Tables 2 and 3**). However, some of the regression cases may be due to decrease of plaque or component volume, rendering them imperceptible on the MRI scans or leading to measurement error.

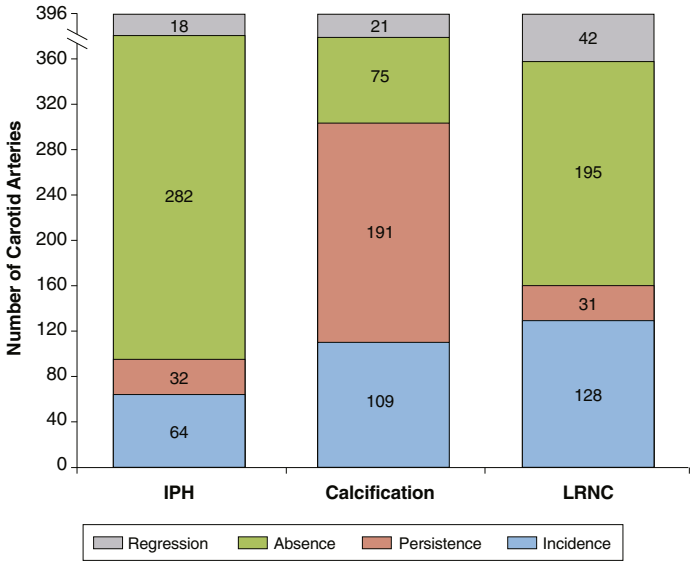
INTRAPLAQUE HEMORRHAGE. We found that age was associated with the development of new IPH. This finding is in line with previous cross-sectional investigations. Previous studies have described the association between age and IPH prevalence, with ORs of 1.08 per 1-year increase (95% CI: 1.02 to 1.14) and 1.8 per 10-year increase (95% CI: 1.60 to 2.10) (17,21). In the present study, antihypertensive drug use and severe hypertension were the strongest independent risk factors in the development of new

TABLE 2 Plaque Characteristics in Men and Women on the Baseline and Follow-Up Scans

	All Arteries	p Value*	Men (n = 226)	Women (n = 170)	p Value†
Plaque presence					
Baseline	352	<0.001	204	148	0.315
Follow-up	378		217	161	0.535
IPH presence					
Baseline	50	<0.001	35	15	0.062
Follow-up	96		62	34	0.100
Calcification presence					
Baseline	212	<0.001	128	84	0.257
Follow-up	300		175	125	0.475
LRNC presence					
Baseline	73	<0.001	46	27	0.325
Follow-up	159		98	61	0.157

Values are numbers of carotid arteries. *For the difference between baseline and follow-up scans. †For the difference between men and women.
IPH = intraplaque hemorrhage; LRNC = lipid-rich necrotic core.

FIGURE 3 Changes in Plaque Components Over a 4-Year Interval (396 Carotid Arteries)



Graph quantifying change in plaque components over time, including incidence, regression, persistence and absence. Each **column** corresponds to a different component; from **left to right**: intraplaque hemorrhage, calcification, and lipid-rich necrotic cores.

TABLE 3 Change in Plaque Composition Over Time in 396 Carotid Arteries

	IPH	Calcification	LRNC
Incidence	64/346 (18.50)	109/184 (59.20)	128/323 (39.60)
Persistence	32/50 (64.00)	191/212 (90.10)	31/73 (42.50)
Absence	282/346 (81.50)	75/184 (40.80)	195/323 (60.40)
Regression	18/50 (36.00)	21/212 (9.90)	42/73 (57.50)
Incidence and/or persistence	80/396 (20.20)	301/396 (76.00)	160/396 (40.40)

Values are n/number at risk (valid %). Valid percentage is calculated based on subjects at risk of developing the outcome at baseline.
See Table 2 for abbreviations.

IPH. Mild hypertension in women and severe hypertension in men were independently associated with IPH incidence. It is likely that patients under antihypertensive medication had more severe hypertension, thus contributing to their greater risk of developing IPH.

IPH is believed to be formed when the endothelial integrity of the intraplaque vessels is compromised, without supporting smooth muscle cells, leading to leakage of erythrocytes from the lumen to the plaque (2,22). Previous investigations have shown that IPH and rupture of the fibrous cap are associated with an increased density of microvessels (2). Thus, considering the pathophysiology of IPH, increased blood pressure and arterial wall damage caused by long-term hypertension can lead to leakage of erythrocytes from the lumen of the artery to the atherosclerotic plaque. This finding also adds to previous cross-sectional studies reporting an association between pulse pressure and hypertension with prevalent IPH (7,17). We found no association in the previous literature between antihypertensive drugs and atherosclerotic plaque composition. Our findings give us insight that either hypertension or antihypertensive drug use may be associated with the development of new IPH. Further studies using longitudinal design, serial MRI images, and larger sample sizes are needed to consolidate this association.

Analyzing different classes of antihypertensive drugs separately may also clarify whether there is a specific mechanism involved in plaque component change that might differ according to antihypertensive type. Due to limited sample size per class of antihypertensive drugs, we did not explore potential differences by types of antihypertensive.

CALCIFICATION. Hypertension was associated with calcification incidence in model 1 only (**Table 1**). Online Table 1 shows the association between hypertension and calcification incidence and/or persistence after multivariate adjustment; this association seems to be related more to mild

TABLE 4 Cardiovascular Risk Factors and Incidence of Plaque Components (in 396 Carotid Arteries)

	Hemorrhage, n = 64 (18.50%)				Calcification, n = 109 (59.20%)				LRNC, n = 128 (39.60%)			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age	1.07	1.03-1.11*	1.07	1.02-1.11*	1.02	0.98-1.06	1.04	1.00-1.09*	0.99	0.96-1.01	1.01	0.98-1.04
Sex	0.71	0.37-1.35	0.92	0.44-1.95	1.08	0.55-2.14	0.87	0.32-2.34	0.73	0.45-1.20	0.48	0.27-0.85*
Diabetes mellitus	0.74	0.31-1.76	0.87	0.33-2.28	11.01	1.36-89.18*	8.73	0.68-111.19	0.60	0.32-1.13	0.69	0.35-1.35
Antihypertensive drug use	2.38	1.24-4.57*	3.87	1.89-7.93*	1.84	0.87-3.90	0.93	0.36-2.37	1.04	0.63-1.73	1.46	0.77-2.77
Systolic blood pressure	1.01	1.00-1.02	1.02	0.99-1.04	1.01	0.99-1.03	0.98	0.95-1.01	0.99	0.98-1.00	0.98	0.96-1.00
Diastolic blood pressure	1.01	0.98-1.04	1.00	0.96-1.04	1.03	1.00-1.06	1.05	0.99-1.10	1.00	0.98-1.02	1.03	1.00-1.07
Hypertension†	1.98	0.86-4.55	2.19	0.94-5.11	2.16	1.07-4.37*	1.90	0.84-4.31	0.78	0.44-1.37	1.01	0.53-1.91
Mild	1.75	0.71-4.33	1.79	0.70-4.59	2.24	1.02-4.94*	2.33	0.91-5.95	0.82	0.44-1.54	1.05	0.53-2.08
Moderate	2.01	0.73-5.53	2.22	0.82-5.99	2.38	0.89-6.38	2.14	0.65-7.06	0.98	0.50-1.93	1.28	0.61-2.68
Severe	3.17	1.20-8.39*	4.72	1.50-14.80*	1.63	0.47-5.63	0.83	0.20-3.46	0.58	0.18-1.82	0.74	0.22-2.54
Total cholesterol	0.97	0.71-1.32	1.03	0.69-1.54	1.10	0.78-1.54	1.39	0.91-2.12	1.38	1.12-1.71*	1.37	1.08-1.74*
HDL cholesterol	0.52	0.20-1.39	0.35	0.11-1.17	0.50	0.18-1.43	0.75	0.26-2.21	1.66	0.94-2.93	1.57	0.79-3.08
Statins Use	1.16	0.58-2.32	0.99	0.41-2.35	1.22	0.51-2.90	1.29	0.40-4.20	0.71	0.42-1.21	1.09	0.60-1.99
Body mass index	0.93	0.85-1.03	0.89	0.79-1.00	1.18	1.07-1.29*	1.11	0.98-1.24	0.97	0.91-1.04	1.01	0.93-1.09
Smoking												
Current vs. never	1.72	0.83-3.59	1.28	0.44-3.71	0.55	0.19-1.62	0.54	0.18-1.64	1.09	0.52-2.26	1.08	0.50-2.32
Past vs. never	1.35	0.46-4.02	1.98	0.87-4.47	0.40	0.17-0.93*	0.32	0.13-0.81*	0.96	0.51-1.79	0.87	0.47-1.61

Model 1: Adjusted for age, sex, and follow-up days. Model 2: Additionally adjusted for baseline wall volume, diabetes (yes/no), smoking (never, past, or current), systolic blood pressure, diastolic blood pressure, use of antihypertensive agents (yes/no), body mass index, total cholesterol, high-density lipoprotein (HDL) cholesterol, and use of statins (yes/no), when appropriate. *$p < 0.05$. †When we analyzed hypertension and hypertension severity, we did not adjust for antihypertensive drug use, systolic blood pressure, or diastolic blood pressure. Blood pressure severity classification was made after adjustment for treatment. When adjusted values would fit normal blood pressure parameters (<140/90 mm Hg), they were considered as "mild hypertension."
CI = confidence interval; OR = odds ratio; LRNC = lipid-rich necrotic core.

hypertension. The association between diabetes and incident calcification was not significant after multivariable adjustments, although this outcome may be related to low statistical power. Classical cardiovascular risk factors have been found to be associated with prevalent arterial calcification in cross-sectional studies (17,23-27). Three studies have shown the association between diabetes mellitus and presence of arterial calcification (23-25). A longitudinal study using CT scans, which considered change in calcification volume, found that age, diabetes mellitus, hypertension, serum glucose levels, and calcification load were predictors for calcification growth. Previous studies have already reported the relation between hypertension and hyperlipidemia and presence of arterial calcification (23,25,26). Our longitudinal study also found results comparable to a previous longitudinal study regarding the association between hypertension and newly detectable calcification loads (27). In contrast to former studies, we found an inverse association between past smoking and calcification incidence (23,25,26). However, in the sex-stratified analysis, this effect was present only in men, who had a stronger smoking history at baseline. This association was also not shown in the incidence and/or persistence analyses. Furthermore, there was no statistically significant relation between current smoking and calcification incidence.

LIPID-RICH NECROTIC CORE. We found a relation between cholesterol levels and LRNC incidence (multivariable adjusted). This association was shown to be more significant in women than in men (Online Table 2). It is already known that carotid plaques with larger LRNC at baseline are associated with a higher risk of plaque rupture over a 3-year follow-up period (28). Former studies have established the relation between higher cholesterol levels and prevalence of LRNC (17,29,30). Likewise, plaques with LRNC have been related to higher risk of cardiovascular events (31). Despite that, the interobserver agreement for LRNC was moderate (0.44; 95% CI: 0.33 to 0.55) in our study, the intra-observer agreement was almost perfect (0.85; 95% CI: 0.74 to 0.96). Our results are comparable to previous findings and indicate that high cholesterol levels are a risk factor for the development of new LRNC (32).

CONCLUSIONS

In these community-dwelling subjects, characteristics of plaque composition changed dramatically within a few years. Our study showed several classical cardiovascular risk factors that were associated with change in carotid plaque composition in community-dwelling subjects. As such, it provides more insight into the pathophysiology of carotid plaques change over time, which eventually may be useful in the prevention of cardiovascular events.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: It is increasingly understood that plaque composition, such as IPH, calcification, and LRNC, are strong predictors of plaque vulnerability to rupture. In carotid plaques, its rupture can lead to thromboembolism and cerebral ischemia. The factors that lead to the development of vulnerable plaques are not yet well determined and may be of great relevance to improve the prevention, treatment, and prediction of CVD. High-resolution MRI recently has enabled accurate, noninvasive, in vivo identification of plaque components.

TRANSLATIONAL OUTLOOK: The determinants of change of carotid plaque components require further investigation, as well as a quantitative measurement of each plaque component.

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KEY WORDS atherosclerosis, calcification, intraplaque hemorrhage, lipid-rich necrotic core, MRI

APPENDIX For the statistical analyses and supplemental tables, please see the online version of this paper.