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Determinants of carotid atherosclerotic plaque burden in a stroke-free population

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ABSTRACT

Background and aims: In a large stroke-free population, we sought to identify cardiovascular risk factors and carotid plaque components associated with carotid plaque burden, lumen volume and stenosis.
Methods: The carotid arteries of 1562 stroke-free participants from The Rotterdam Study were imaged on a 1.5-Tesla MRI scanner. Inner and outer wall of the carotid arteries were automatically segmented and lumen volume (mm^3), wall volume (outer wall–inner wall) and plaque burden (wall volume/outer wall volume) (%) were quantified. Plaque components were visually determined and luminal stenosis was assessed. We analyzed associations of cardiovascular risk factors and carotid plaque components with plaque burden and lumen volumes using regression analysis.
Results: We investigated 2821 carotid plaques and found that women had larger plaque burden ($50.7 \pm 7.8\%$ *vs.* $49.2 \pm 7.7\%$, $p < 0.0001$) and smaller lumen volumes (933 ± 286 mm^3 *vs.* 1078 ± 334 mm^3, $p < 0.0001$) than men. In women, age, HDL-cholesterol and systolic blood pressure, and in men, total cholesterol, non-HDL cholesterol and statin use were independently associated with higher plaque burden and lumen volume. Furthermore, smoking and diabetes were associated with lumen volume in men (respectively $p = 0.04$ and $p = 0.002$). Intraplaque hemorrhage (IPH) and lipid were related to a larger plaque burden (OR 1.30 [1.05–1.60] and OR 1.28[1.06–1.55]). Finally, within the highest quartile of plaque burden, IPH was strongly associated with luminal stenosis independent of age, sex, plaque burden and composition (Beta = 15.2; [11.8–18.6]).
Conclusions: Several cardiovascular risk factors and plaque components, in particular IPH, are associated with higher plaque burden. Carotid IPH is strongly associated with an increased luminal stenosis.
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1. Introduction

Subclinical carotid atherosclerosis is highly prevalent among the elderly and is a common cause of cerebrovascular disease. Direct non-invasive measurements of the burden and composition of asymptomatic atherosclerotic plaque have, therefore, been proposed as a way to improve cardiovascular disease prevention and management. Plaque burden may provide more information on the extent of atherosclerosis beyond luminal stenosis [1,2]. Furthermore, it has been postulated that plaque burden is associated with an increased risk of stroke and coronary heart disease [3–7].

Different imaging techniques have been used to quantify and monitor carotid plaque burden and luminal stenosis. Studies that used ultrasound to assess carotid plaque burden are limited, because of the low contrast-to-noise ratio of US for the detection of plaque components [8]. Whereas computed tomography has been

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; IPH, intraplaque hemorrhage; MRI, magnetic resonance imaging.

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validated for carotid calcium quantification, its application for detection of soft non-calcified plaques remains limited. Magnetic resonance imaging (MRI) depicts the plaque composition as well as the inner and outer wall of the entire carotid artery and is non-invasive in nature. Hence, MRI may be a promising modality to investigate atherosclerotic plaque burden in the general population. Though some previous studies investigated plaque volume on MRI using manual segmentation, these studies were mostly conducted in selected or small samples [9–11]. Semi-automated segmentation of atherosclerotic plaque may provide a fast, accurate and reproducible tool for large-scale application [12,13].

Information on determinants of plaque burden and lumen volume in the general population remains scarce. In addition, it is still unclear how (vulnerable) plaque components relate to overall plaque burden and lumen reduction. Therefore, we quantified carotid atherosclerotic plaque burden and lumen volume in a large population of stroke-free subjects and determined sex-specific associations with cardiovascular risk factors and carotid plaque composition. In addition, we evaluated the role of carotid plaque components in the development of luminal stenosis.

2. Materials and methods

2.1. Study population

The study is part of the Rotterdam Study, a large population-based cohort study, which was designed to investigate determinants of various diseases among the elderly. 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. A written informed consent was obtained from all participants. All inhabitants of Ommoord, a suburb of Rotterdam, aged 45 years and over were invited. The rationale and design of the Rotterdam Study have been described elsewhere [14]. As part of the baseline examinations, all participants underwent ultrasonography to measure carotid wall thickness and underwent carotid MRI if wall thickness was ≥2.5 mm in at least one carotid artery (n = 1982 subjects, 74% of those initially invited) [15]. Participants who had a past medical history positive for stroke (defined as a history of ischemic or unspecified stroke but not hemorrhagic stroke) or TIA were excluded (n = 95). In addition, MRI scans were excluded if visual quality was insufficient for assessment of plaque composition (n = 113) or if scanning was interrupted due to claustrophobia (n = 106). We further excluded carotid arteries without atherosclerotic plaque or if wall thickness was <2 mm on MRI (n = 284). This resulted in a total of 3052 carotid arteries in 1668 scanned participants available for analysis.

2.2. Carotid MRI scanning and visual analysis of plaque characteristics

Imaging was performed with a 1.5 Tesla scanner (GE Healthcare, Milwaukee, WI, USA) with a bilateral phased-array surface coil (Machnet, Eelde, the Netherlands). A standard scanning protocol was used with a total scanning time of approximately 30 min. The carotid MRI protocol, reading and reproducibility are described elsewhere, and include four 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 resolution; a PDw-echo planar imaging (EPI) sequence, and a T2w-EPI sequence and two 3D sequences: a 3D-T1w-gradient echo (GRE) sequence and a 3D phased-contrast MR angiography [16]. We evaluated both carotid arteries and assessed plaque characteristics in all plaques with a maximum thickness of ≥2.0 mm on MRI. On the PDw-FSE-BB images, maximum carotid wall thickness was measured and degree of luminal stenosis was calculated using the North American Symptomatic Carotid Endarterectomy Trial NASCET criteria [17]. Plaques were visually reviewed for the presence of three different plaque components; presence of intraplaque hemorrhage (IPH), lipid core and calcification as described previously and multiple components were permitted within one plaque [15].

2.3. Automated segmentation of lumen volume and plaque burden

The MRI-based automated method for carotid lumen and wall volume quantification, as described by Van 't Klooster et al. [13] and Hameeteman et al. [12] was used. The method showed comparable results to the manual annotations in terms of wall volume and normalized wall index measurements and can therefore be used to replace the manual annotations. Using the PDw-FSE-BB sequence and 3D phased-contrast MR image, the method automatically quantifies the lumen volume and plaque burden of the carotid artery in a region around the bifurcation. Briefly, the method combines lumen and outer wall segmentation based on deformable model fitting with a learning-based segmentation correction step. In order to ensure a similar coverage among carotid arteries, we included segments of approximately 25 mm length in central vessel axis centered at the bifurcation point; i.e. 12.5 mm segment length on both sides from the bifurcation point to the common and internal carotid artery. The bifurcation point was manually annotated and defined as the first transversal plane on which two separate lumens (one of the internal carotid artery and one of the external carotid artery) are visible. Volumes of the lumen (mm^3) and outer vessel wall of the carotid arteries were obtained using a custom-designed automatic image analysis tool [12], thus eliminating the need for manual contouring. User input was limited to four seed points in the common, internal and external carotid artery and bifurcation. Wall volume was calculated as the difference between the outer vessel wall volume and lumen volume. Plaque burden was operationalized by measuring wall volume/outer vessel wall volume and multiplied by 100%.

In total, 2923 extracted volumes of the lumen and outer vessel wall were available. Missing extracted volumes (n = 129, (7.6%)) were due to incomplete MRI data (n = 50), failure of image registration between PDw-FSE-BB and phase-contrast MRA sequences (n = 20) or failure of automated segmentation (n = 59). Based on visual inspection, 3 outliers were removed because of suboptimal segmentation, and volumes that are further than three standard deviations from the mean were also considered as outliers and removed (n = 99). In total, n = 2821 carotid arteries (in n = 1562 individuals) were used for the statistical analyses.

2.4. Covariates

Smoking status was classified as ever or never. Serum high-density lipoprotein (HDL)-cholesterol (mmol/L) and total cholesterol (mmol/L) were measured using standard laboratory techniques. Non-HDL cholesterol (mmol/L) was calculated as the difference between total and HDL-cholesterol. Body mass index (BMI) [weight/height^2], expressed in kg/m^2, was determined for all subjects. Blood pressure (mmHg) was measured with a random-zero sphygmomanometer after 5 min of rest with the subject in a sitting position and the mean of the two blood pressure values was used. Diabetes mellitus was defined as a fasting blood glucose >7.0 mmol/L, non-fasting glucose >11.0 mmol/L, or use of antidiabetic medication. Hypertension was defined as a blood pressure >140/90 mmHg or the use of antihypertensive medication. Medication dispensing data were obtained from the fully computerized pharmacies in the Ommoord suburb. Information on all filled

Table 1
Baseline characteristics of 1562 study participants.

Variable	Overall	Men (839)	Women (723)	*p*-value
Age (years)	72.7 ± 9.2	72.1 ± 8.9	73.4 ± 9.4	0.005
Smoking (ever) (%)	73.6	85.0	60.4	<0.001
HDL-cholesterol (mmol/L)	1.4 (0.4)	1.3 (0.3)	1.6 (0.4)	<0.001
Total cholesterol (mmol/L)	5.7 (1.0)	5.4 (1.0)	5.9 (1.0)	<0.001
Non-HDL cholesterol (mmol/L)	4.3 (1.0)	4.2 (1.0)	4.4 (1.0)	<0.001
BMI (kg/m^2)	27.3 (3.6)	27.4 (3.1)	27.1 (4.0)	0.1
Systolic blood pressure (mmHg)	145 (21)	145 (20)	145 (22)	0.8
Diastolic blood pressure (mmHg)	80 (11)	82 (11)	79 (11)	<0.001
Diabetes (%)	13.9	16.4	10.9	0.002
Hypertension (%)	74.5	75.1	73.9	0.7
Antihypertensive drugs (%)	39.7	40.3	39.0	0.6
Statins (%)	27.7	32.1	22.7	<0.001

Values are percentages or means (SD).
p-value is for age-adjusted differences between men and women.
(Non)-HDL, high-density lipoprotein; BMI, body mass index.

prescriptions of antihypertensive drugs and lipid lowering drugs on date of carotid MRI scans was available.

2.5. Data analysis

Lumen volume and plaque burden were normalized for carotid artery length and adjusted for age. Sex-specific relationships of cardiovascular risk factors with plaque burden and lumen volume were assessed by means of generalized estimation equation with an unstructured working correlation matrix including two levels per participant, namely the left and right carotid artery. Continuous variables were expressed per standard deviation (SD) and plaque burden and lumen volumes were transformed to a standardized Z-score in order to allow comparison across these measures. Adjustments were made for age and carotid segment length (model 1), and additionally for smoking, HDL-cholesterol, total cholesterol, BMI, systolic blood pressure, diastolic blood pressure, diabetes mellitus, hypertension, antihypertensive drug use and statin use (model 2). Non-HDL was additionally adjusted for the same covariates as in model 2, but without HDL-cholesterol and total cholesterol (model 3).

In secondary analysis, we categorized lumen volume and plaque burden using sex-specific quartiles and compared the lowest quartile in lumen volume and the highest quartile of plaque burden to the other three quartiles, respectively. In addition, we studied the odds ratio (OR) for prevalence of different plaque components (i.e., hemorrhage, calcification and lipid) in relation to lowest quartile of lumen volume and the highest quartile of plaque burden and we tested for effect modification by sex. Finally, within each quartile of plaque burden, we investigated the association of all plaque components with luminal stenosis. Longitudinal coverage of carotid arteries was not equal for all MRI scans. In order to evaluate the effect of variation in carotid segment length, we additionally performed a sensitivity analysis for which we excluded carotid arteries with a segment length <25 mm. All analyses were carried out using SPSS Statistical Package version 20.0 (Chicago, IL, USA).

3. Results

Table 1 describes the baseline characteristics of the study population. In a total of 1562 individuals, mean age was 72.7 ± 9.2 years and 723 (46%) were women. Women were slightly older, smoked less, had higher HDL-cholesterol, total cholesterol and non-HDL cholesterol values, lower diastolic blood pressure, less often diabetic and used less statins (age-adjusted *p*-values <0.002). In comparison to men, women had smaller lumen volumes (933 ± 286 mm^3 *vs.* 1078 ± 334 mm^3 in men, $p < 0.0001$) and larger plaque burden (50.7 ± 7.8% *vs.* 49.2 ± 7.7% in men, $p < 0.0001$).

In Table 2A and B, we reported the sex-specific associations between cardiovascular risk factors, lumen volume and plaque burden after adjustment for age and segment length (model 1) as well as all other cardiovascular risk factors (model 2 or 3). In women, younger age, lower HDL-cholesterol and higher systolic blood pressure were independently associated with larger plaque burden and smaller lumen volume, whilst in men, higher total cholesterol and non-HDL cholesterol levels and statin use were related to higher plaque burden and lower lumen volume. Also, ever smoking and diabetes were associated with lumen volume in men.

Lipid, IPH and calcifications were present in 28,6%, 21,5% and 70,8% of the 2821 carotid atherosclerotic plaques. Table 3 shows the association of the various plaque components with low lumen volume (lowest quartile *vs.* others) and high plaque burden (highest quartile *vs.* others). With respect to lumen volume or plaque burden, there was no interaction between sex and plaque component (data not shown), therefore we did not stratify for sex. In sex and age-adjusted analysis, IPH and lipid were significantly associated with low lumen volume (OR 1.42 [1.15–1.75] and OR 1.34 [1.12–1.62], respectively) and with high plaque burden (OR 1.30 [1.05–1.60] and OR 1.28 [1.06–1.55], respectively).

Finally, we investigated the relation between plaque components and luminal stenosis across the four quartiles of plaque burden (Fig. 1, Table 4). Fig. 1 shows a graphical distribution of the mean luminal stenosis in carotid arteries conditioned on plaque composition per quartile of plaque burden. Table 4 shows that IPH yielded the strongest association with luminal stenosis, independent of age, sex, plaque burden and the other plaque components in all quartiles of plaque burden.

The sensitivity analysis for which we excluded carotid arteries with a segment length <25 mm (n = 503 (16.7%)) on MRI, resulting in a total of n = 2500 carotid arteries (n = 1553 individuals) did not materially lead to different results (Supplemental Tables 1 and 2).

4. Discussion

In this population-based study in stroke-free subjects, we found that several cardiovascular risk factors are associated with carotid plaque burden and lumen volume as measured on MRI. Younger age, lower HDL-cholesterol levels and higher systolic blood pressures were associated with larger plaque burden and smaller lumen volume in women, whilst in men, significant associations were observed with total cholesterol and non-HDL cholesterol levels and statin use. Furthermore, we found IPH and lipid to be associated with low lumen volume and high plaque burden. Across all

Table 2
Cardiovascular determinants for lumen volume and plaque burden. (A) In women, (B) in men.

Variable	Z-score			Z-score		
	Lumen volume			Plaque burden		
	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value
(A)						
Model 1						
Age	0.103	[0.051; 0.156]	<0.001	−0.085	[−0.142; −0.027]	0.004
Smoking (ever)	0.062	[−0.005; 0.130]	0.07	−0.049	[−0.126; 0.028]	0.2
HDL-cholesterol	0.070	[0.018; 0.122]	0.008	−0.075	[−0.131; −0.019]	0.008
Total cholesterol	−0.028	[−0.082; 0.026]	0.3	0.003	[−0.060; 0.065]	0.9
Non-HDL cholesterol	−0.051	[0.101; −0.002]	0.04	0.030	[−0.029; 0.089]	0.3
BMI	−0.009	[−0.055; 0.038]	0.7	0.031	[−0.020; 0.082]	0.2
Systolic blood pressure	−0.026	[−0.084; 0.033]	0.4	0.044	[−0.016; 0.105]	0.1
Diastolic blood pressure	0.020	[−0.039; 0.078]	0.5	−0.012	[−0.079; 0.055]	0.7
Diabetes	−0.043	[−0.226; 0.140]	0.6	0.086	[−0.094; 0.265]	0.3
Hypertension	0.040	[−0.098; 0.179]	0.6	−0.020	[−0.160; 0.119]	0.8
Antihypertensives	0.009	[−0.111; 0.130]	0.9	0.010	[−0.118; 0.138]	0.9
Statins	−0.087	[−0.212; 0.038]	0.2	0.045	[−0.100; 0.190]	0.5
Model 2						
Age	0.134	[0.066; 0.203]	<0.001	−0.118	[−0.187; −0.048]	0.001
Smoking (ever)	0.067	[−0.003; 0.137]	0.06	−0.053	[−0.131; 0.025]	0.2
HDL-cholesterol	0.081	[0.024; 0.137]	0.005	−0.075	[−0.134; −0.015]	0.01
Total-cholesterol	−0.038	[−0.093; 0.017]	0.2	0.014	[−0.050; 0.78]	0.7
Non-HDL cholesterol[3]	−0.037	[−0.090; 0.016]	0.2	0.013	[−0.049; −0.076]	0.7
BMI	0.0004	[−0.052; 0.052]	1.0	0.019	[−0.034; 0.073]	0.5
Systolic blood pressure	−0.094	[−0.175; −0.014]	0.02	0.112	[0.029; 0.196]	0.009
Diastolic blood pressure	0.065	[−0.011; 0.141]	0.09	−0.068	[−0.156; 0.020]	0.1
Diabetes	0.008	[−0.180; 0.195]	0.9	0.024	[−0.163; 0.210]	0.8
Hypertension	0.107	[−0.059; 0.272]	0.2	−0.125	[−0.308; 0.058]	0.2
Antihypertensives	0.020	[−0.118; 0.157]	0.8	−0.013	[−0.158; 0.131]	0.9
Statins	−0.087	[−0.225; 0.050]	0.2	0.023	[−0.135; 0.180]	0.8
(B)						
Model 1						
Age	−0.013	[−0.077; 0.052]	0.7	−0.011	[−0.070; 0.048]	0.7
Smoking (ever)	0.087	[0.00; 0.174]	0.050	−0.022	[−0.104; 0.061]	0.6
HDL-cholesterol	0.055	[−0.024; 0.134]	0.2	−0.030	[−0.106; 0.047]	0.4
Total cholesterol	−0.022	[−0.083; 0.039]	0.5	0.048	[−0.007; 0.103]	0.09
Non-HDL cholesterol	−0.039	[−0.100; 0.022]	0.2	0.058	[0.003; 0.113]	0.04
BMI	−0.034	[−0.106; 0.037]	0.3	0.059	[−0.011; 0.128]	0.1
Systolic blood pressure	0.001	[−0.069; 0.070]	1,0	−0.023	[−0.086; 0.040]	0.5
Diastolic blood pressure	0.018	[−0.045; 0.081]	0.6	−0.037	[−0.095; 0.020]	0.2
Diabetes	−0.270	[−0.422; −0.119]	<0.001	0.131	[−0.007; 0.268]	0.06
Hypertension	−0.045	[−0.199; 0.109]	0.6	−0.036	[−0.174; 0.102]	0.6
Antihypertensives	−0.022	[−0.150; 0.107]	0.7	−0.062	[−0.179; 0.055]	0.3
Statins	−0.158	[−0.293; −0.024]	0.02	0.112	[−0.011; 0.235]	0.07
Model 2						
Age	−0.004	[−0.080; 0.073]	0.9	−0.015	[−0.083; 0.053]	0.6
Smoking (ever)	0.092	[0.003; 0.181]	0.04	−0.034	[−0.118; 0.050]	0.4
HDL-cholesterol	0.040	[−0.045; 0.125]	0.4	−0.026	[−0.108; 0.057]	0.5
Total cholesterol	−0.073	[−0.140; −0.006]	0.03	0.071	[0.012; 0.130]	0.02
Non-HDL cholesterol[3]	−0.072	[−0.136;−0.008]	0.03	0.069	[0.012; 0.126]	0.02
BMI	−0.018	[−0.094; 0.059]	0.6	0.058	[−0.017; 0.133]	0.1
Systolic blood pressure	−0.013	[−0.108; 0.081]	0.8	0.008	[−0.076; 0.091]	0.9
Diastolic blood pressure	0.037	[−0.044; 0.118]	0.4	−0.049	[−0.125; 0.027]	0.2
Diabetes	−0.262	[−0.426; −0.098]	0.002	0.134	[−0.011; 0.278]	0.07
Hypertension	−0.053	[−0.249; 0.143]	0.6	0.006	[−0.174; 0.186]	0.9
Antihypertensives	0.057	[−0.087; 0.200]	0.4	−0.116	[−0.247; 0.014]	0.08
Statins	−0.155	[−0.302; −0.009]	0.04	0.163	[0.031; 0.296]	0.02

Values are adjusted mean differences (95% confidence interval) in lumen volume and plaque burden, expressed in Z-scores, per increase in SD or presence of each determinant.
Model 1 adjusted for age and segment length.
Model 2 adjusted for cardiovascular risk factors (smoking, HDL-level, total cholesterol, BMI, systolic blood pressure, diastolic blood pressure, diabetes mellitus, hypertension, antihypertensive drug use and statin use).
Model 3 same as model 2 but without HDL-cholesterol and total cholesterol.
(Non)-HDL, high-density lipoprotein; BMI, body mass index. CI, confidence interval.

categories of plaque burden, presence of IPH, lipid and calcification was related to more severe luminal stenosis, however, this was most pronounced for IPH.

To our knowledge, this is the largest study to date to take advantage of MRI-based automated segmentation to quantify plaque burden and lumen volumes in a stroke-free population. All participants were preselected on presence of advanced atherosclerotic plaque on ultrasound. Bilateral plaque burden characterization was achieved with minimal user interaction. The longitudinal coverage of 25 mm in central vessel axis permitted by the automated segmentation allows detection of atherosclerotic plaques in the common and internal carotid artery, which can be potentially missed with ultrasound or computed tomography imaging. Restricting our study to a stroke-free population allowed us

Table 3
Association of different plaque components with low lumen volume (lowest quartile *vs.* other three quartiles), high plaque burden (highest quartile *vs.* other three quartiles).

	Low lumen volume			High plaque burden		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
IPH	1.42	[1.15; 1.75]	0.001	1.30	[1.05; 1.60]	0.02
Lipid	1.34	[1.12; 1.62]	0.002	1.28	[1.06; 1.55]	0.009
Calcification	0.92	[0.76; 1.11]	0.4	1.02	[0.84; 1.25]	0.8

Age and sex-adjusted.
OR, odds ratio; CI, confidence interval; IPH, intraplaque hemorrhage.

to generalize our findings to asymptomatic individuals. All analyses that had enough power to do so were performed stratified on sex, because atherosclerosis distributions are known to be different amongst men and women.

Although the automatic quantification of atherosclerosis was highly reproducible, this step remains difficult, because of the limited tissue contrast between the outer vessel wall and the surrounding soft tissues, limiting the accuracy of outer vessel wall contouring. Furthermore, the automatic tool is not able to differentiate between the intimal thickening and the media and adventitia. Both limitations may have resulted in a systematic overestimation of plaque volume measurements, which we expect to be non-differential. We found no clear definitions of plaque burden measurement in the literature as different imaging techniques have previously been used to quantify plaque burden [18]. In our study, we decided to operationalize plaque burden using wall volume (outer vessel wall volume − lumen volume), relative to the outer vessel volume, normalized to carotid artery length, as this is a practical approach to evaluate, this value across a large number of study subjects. Nevertheless, it must be noted that our findings cannot be directly compared to the various studies on this issue. Our cross-sectional study design restricted our interpretation of the data with respect to cause and consequence. For this reason, prospective longitudinal studies should investigate the association of cardiovascular risk factors with plaque progression.

Previous studies using different imaging modalities like ultrasound or computed tomography have demonstrated the potential to use plaque burden measurements as a direct measure of the extent and severity of atherosclerotic disease [19]. Plaque measurements in these studies have varied between estimation of the intima medial thickness and luminal stenosis on ultrasound to measurement of calcified plaque burden on CT [1,20,21]. Nevertheless, studies that investigated the association of cardiovascular risk factors in relation to plaque burden measurements using MRI remain scarce. In the previous studies, independent associations were detected between plaque burden and smoking, HDL-cholesterol, total cholesterol, non-HDL cholesterol and systolic blood pressure [1,20,21]. In 1670 subjects from the general population, the Atherosclerosis Risk in Communities (ARIC) study demonstrated that carotid plaque burden, automatically measured on MRI, was associated with atherogenic cholesterol, such as total cholesterol, non-HDL cholesterol and low HDL-cholesterol levels [21]. In concordance to these findings, our study showed significant associations in both sexes between atherogenic cholesterol and higher plaque burden and smaller lumen volume.

In addition, whereas sex differences regarding the severity of atherosclerosis are widely known, several studies confirm that plaque composition as well as outcomes in vascular disease differ between sexes [22–25]. In our study, it is possible that a higher relative plaque burden in women may be partly explained by the smaller artery size in women than in men, whereas absolute amount of plaque may be equal or less than in men. Nevertheless, our study underlines the importance of sex-differentiated management in patients with asymptomatic carotid atherosclerosis across all stages of carotid stenosis.

When examining plaque composition, we found lipid and IPH to be independently associated with low lumen volume and high plaque burden. These findings are consistent with results from prior studies that investigated the relation of plaque composition to plaque volumes and in which high plaque volume was often associated with a large lipid core [26–28]. Although these studies provide insight on the association between plaque components and plaque burden in more advanced atherosclerosis, none considered the role of IPH. When we took into account differences in total plaque burden, we found that persons with IPH had the largest degree of stenosis compared to persons with lipid or calcification. These findings suggest that the presence of IPH is associated with more severe luminal narrowing independent of the size of the plaque. One hypothesis could be that IPH develops so fast that it overrules preservation of luminal patency by outward remodeling. In our data, this hypothesis is supported by the fact that the outer vessel wall volume of carotid arteries with IPH is significantly

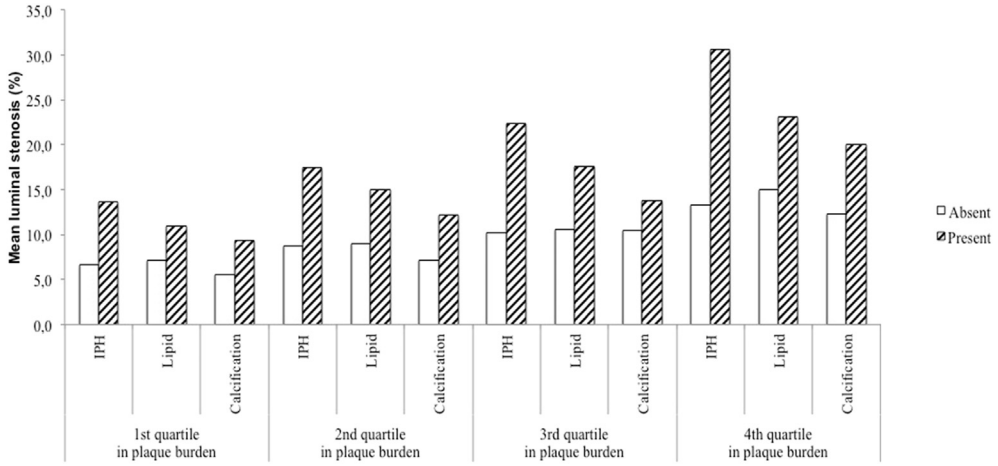

Fig. 1. Mean luminal stenosis in carotid arteries conditioned on plaque composition per quartile of plaque burden. All carotid arteries with the condition are associated with a significantly larger luminal stenosis than carotid arteries without the condition (adjusted for age, sex, plaque burden, segment length) $p \leq 0.005$. IPH, intraplaque hemorrhage.

Table 4
Risk of luminal stenosis with presence of different plaque components stratified on quartile of plaque burden.

Plaque burden	Intraplaque hemorrhage			Lipid			Calcification		
	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value
1st quartile	6.0	[3.6; 8.4]	<0.001	3.0	[0.9; 5.1]	0.005	2.4	[0.2; 4.6]	0.03
2nd quartile	6.8	[4.3; 9.3]	<0.001	5.1	[2.8; 7.3]	<0.001	4.4	[2.1; 6.6]	<0.001
3rd quartile	10.6	[7.7; 13.4]	<0.001	5.6	[3.2; 8.1]	<0.001	3.3	[0.9; 5.8]	0.008
4th quartile	15.2	[11.8; 18.6]	<0.001	6.8	[3.7; 9.9]	<0.001	5.3	[1.9; 8.6]	0.002

Linear regression analysis: all models include sex, age, segment length, plaque burden, lipid, IPH, calcification. Beta indicates risk of luminal stenosis (dependent variable) with presence of different plaque components stratified on quartile of plaque burden.
CI, confidence interval.

smaller than those without IPH when adjusted for age, sex, segment length and plaque burden (1943 mm^3 vs 1996 mm^3; $p < 0.001$). Our findings are also consistent with the findings of Takaya et al. who showed that lesions with IPH at baseline had a greater increase in wall volume and reduction in lumen volume compared with arteries without IPH after eighteen months of follow up [29]. It is well established in the literature that the IPH component is one of the critical factors of the vulnerable carotid artery plaque [30,31]. Although it was suggested that the increased cardiovascular risk of IPH is due to plaque destabilization with thromboembolic complications [32,33], we now show that IPH also strongly contributes to lumen narrowing, in the context of equal plaque burden.

Current screening and diagnostic methods are insufficient to identify the plaques that have a high rupture risk and thus to adequately select individuals who are most likely to suffer from an ischemic stroke. Although still challenging, future research should test the hypothesis whether assessment of plaque burden and (vulnerable) plaque components, as opposed to simple stenosis measurements with carotid ultrasound, may contribute to optimized risk classification and treatment strategies.

Sex-specific cardiovascular risk factors, such as age, HDL-cholesterol, total cholesterol, non-HDL cholesterol, systolic blood pressure, smoking, diabetes and statin use, and plaque composition are associated with plaque burden and lumen volume. Amongst different plaque components, IPH yields the strongest association with luminal stenosis. Hence, the control of modifiable risk factors can prove beneficial in lowering plaque burden. However, prospective longitudinal studies should investigate whether these cardiovascular risk factors and plaque components are also associated with plaque progression.

Conflict 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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Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Selwaness had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.10.030.

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