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ORIGINAL PAPER

Plasma lipidomics and coronary plaque changes: a substudy of the SMARTool clinical trial

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Aims

To date, no studies have investigated the association between lipid species and coronary plaque changes over time, quantitatively assessed by serial imaging. We aimed to prospectively determine the association between lipid species quantified by a plasma lipidomic analysis and coronary plaque changes according to composition assessed by a quantitative serial analysis of coronary computed tomography angiography (CTA).

Methods and results

Patients with suspected coronary artery disease (CAD) undergoing baseline coronary CTA were prospectively enrolled by seven EU centres in the SMARTool study and submitted to clinical, molecular, and coronary CTA re-evaluation at follow-up (an inter-scan period of 6.39 ± 1.17 years). Out of 202 patients who were analysed in the SMARTool main clinical study, a lipidomic analysis was performed in 154 patients before the baseline coronary CTA, and this group was included in the present study. A quantitative CTA analysis was performed by using a separate core laboratory blinded from clinical data. In the univariable analysis, it was found that no lipid species were significantly associated with annual total and calcified plaque changes. After adjusting for clinical variables at baseline and statin use, it was found that three lipid species were significantly associated with non-calcified plaque progression. In detail, cholesteryl ester(20:3), sphingomyelin (SM)(40:3), and SM(41:1) were found to be positively related to non-calcified plaque progression (Bonferroni-adjusted *P*-values = 0.005, 0.016, and 0.004, respectively).

Conclusion

The current study showed an independent relationship between specific lipid species determined by a plasma lipidomic analysis and non-calcified coronary plaque progression assessed by a serial, quantitative coronary CTA analysis.

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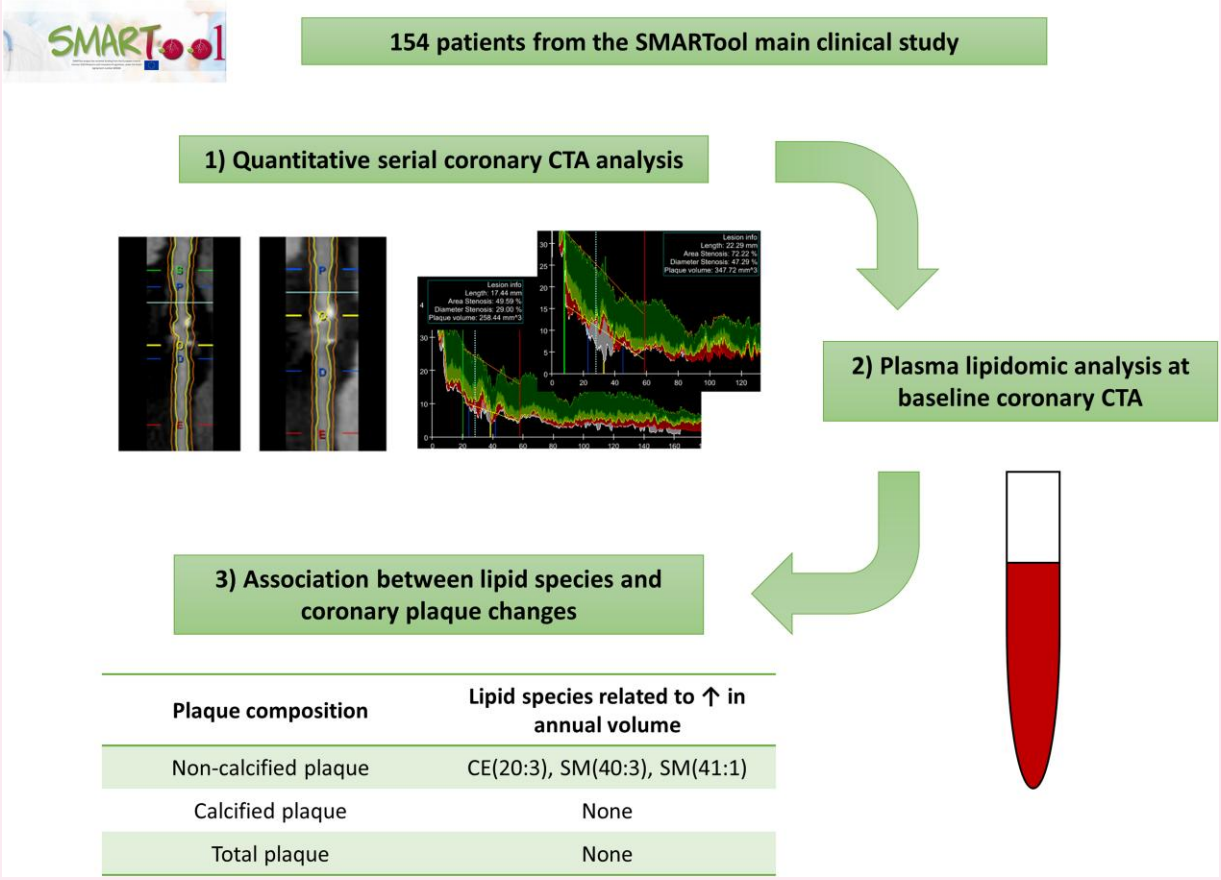
Graphical Abstract



Keywords lipid species • coronary artery disease • coronary plaque progression • non-invasive coronary imaging • coronary computed tomography angiography

Introduction

Coronary artery disease (CAD) remains an important contributor to morbidity and mortality worldwide.[1] Despite the availability of numerous effective management strategies for primary prevention of CAD, models to risk-stratify patients in clinical practice are far from accurate. These models, which are mainly based on clinical history, risk factors, and plasma cholesterol levels, guide the clinician's decision to adopt specific management strategies. Ideally, a non-invasive screening test would help improve patient risk stratification by a simple assessment of circulating lipid species. Among lipids, known to play an important role in CAD formation and composition, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol are often used for this purpose. Recently, nearly 600 other lipid molecular species have been identified in human plasma by a lipidomic analysis.[2] So far, several studies have demonstrated the ability of specific lipid species to predict the burden of non-calcified coronary plaque and necrotic core tissue.[3,4] Interestingly, non-calcified plaques are thought to be at a higher risk of rupture, thereby potentially triggering an acute coronary syndrome.[5–8] Recently, we have demonstrated, by serial computed tomography angiography (CTA) imaging of stable CAD patients on optimal medical therapy, that statin use is associated with an increased progression of calcified coronary plaque and a reduced progression of non-calcified coronary plaque.[9] In addition, multiple studies have shown a relationship between specific lipid species and adverse cardiac outcomes, irrespective of clinical risk factors and cholesterol levels.[4,10,11] However, to date, no studies have investigated the association between lipid species and changes in coronary plaque composition over time, quantitatively assessed by serial imaging. Therefore, in our study, we aimed to prospectively determine the association between lipid species quantified by a plasma lipidomic analysis and coronary plaque changes assessed by a quantitative serial analysis of coronary CTA.

Methods

Patient inclusion

Patients were enrolled from the Horizon 2020–funded PN689068-SMARTool (Simulation Modelling of coronary Artery disease: a tool for clinical decision support) clinical study. In brief, patients with suspected CAD undergoing baseline coronary CTA as part of the EVINCI (Evaluation of Integrated Cardiac Imaging for the Detection and Characterization of Ischaemic Heart Disease; FP7-222915) or the ARTreat (FP7-224297) studies were prospectively enrolled by seven EU centres in the SMARTool study and submitted to clinical, molecular, and coronary CTA re-evaluation at follow-up (an inter-scan period of 6.39 ± 1.17 years). The study design of the SMARTool clinical trial has been described in detail previously and was approved by the local ethical committees of all participating centres (ClinicalTrial.gov Identifiers NCT0444869).[9,12,13] Out of 202 patients

who were analysed in the SMARTool main clinical study, a lipidomic analysis was performed in 154 patients before the baseline coronary CTA, and this group was included in the present study.

Quantitative CTA analysis

A CTA analysis was performed by using a separate core laboratory (Leiden University Medical Center) blinded from clinical data. The complete workflow of a quantitative CTA analysis as performed in the SMARTool clinical trial has been described previously.[9] In short, baseline and follow-up coronary CTA images were first evaluated visually to assess the presence and location of coronary plaques. Coronary arteries were assessed according to the modified 17-segment American Heart Association classification.[14] A CAD score was calculated for all CTAs, based on a comprehensive CTA score incorporating the presence, extent, severity, location, and composition of CAD.[15] Only scan pairs from patients with ≥1 visually assessed coronary plaque at follow-up CTA underwent subsequent quantitative analysis. The quantitative CTA analysis was performed using a dedicated software package (QAngio CT Research Edition version 3.1.2.0). A 3D coronary tree was extracted from the coronary CTA dataset, and straightened multiplanar reconstructions were obtained from each coronary artery. Lumen and vessel wall contours, which were automatically detected by the software package, could be manually adjusted. In addition, reference areas were selected proximally and distally from the coronary lesions based on the assumed normal tapering of the coronary artery. Baseline and follow-up coronary CTAs were analysed side-by-side, and coronary plaques were matched using specific landmarks, such as side branches and distance from the ostium. Plaque composition was determined using predefined intensity cut-off values in Hounsfield units (HUs): −30 to 350 HU for non-calcified plaque and >350 HU for calcified plaque. Total, calcified, and non-calcified plaque volumes were assessed on a per-patient basis by summation of the plaque volumes of individual coronary plaques. Plaque changes were determined by the absolute difference in plaque volume per year using the following formula: (plaque volume at follow-up−plaque volume at baseline)/inter-scan period. Plaque progression was defined as an absolute increase in annual plaque volume (i.e. >0 mm^3 change in plaque volume per year). In contrast, plaque regression was defined as an absolute decrease in annual plaque volume (i.e. <0 mm^3 change in plaque volume per year).

Plasma sample preparation and high-performance liquid chromatography-tandem mass spectrometry–targeted lipidomic analysis

Plasma samples stored at −80°C were thawed at room temperature and immediately subjected to lipid extraction and analysis. Total lipid extraction from an aliquot of plasma was performed according to the Folch procedure: 50 µL of sample were put in a 1.5-mL microcentrifuge tube and diluted with 100 µL of 150-mM NaCl aqueous solution and 600 µL of 0.0625-µM *N*, *N*-dimethylsphingosine (d18:1) in MeOH/$CHCl_3$ 1/2 v/v.[16] The biphasic solution thus formed was incubated at 25°C for 30 min at 1000 rpm in a Thermomixer Compact (Eppendorf, Hamburg, Germany) and then centrifuged at 13 000 rpm for 10 min at 10°C in a Microcentrifuge Heraeus Biofuge Fresco (Thermo Scientific, Waltham, MA, USA). From each sample, the lower phase was transferred into glass vials for the subsequent high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. Lipid species absolute concentrations were obtained using a selected reaction monitoring–based HPLC-MS/MS method and external calibration curves, as previously reported in Michelucci *et al.*[17]

Statistical analysis

The distribution of continuous data was analysed using histograms and Q–Q plots. Continuous variables were displayed as mean ± standard deviation (SD) and compared using the independent Student's *t*-test if normally distributed or the Mann–Whitney *U* test if non-normally distributed. Categorical data were displayed as numbers and percentages and compared using the χ^2 test. The Wilcoxon signed rank test and McNemar test were used for the comparison of paired data. First, a univariable linear regression analysis was performed to assess the association between lipid species at baseline and coronary plaque changes as a continuous variable. The Bonferroni correction method was used to account for multiple comparisons. Second, a multivariable analysis was performed adjusting for cardiovascular risk factors (i.e. age, gender, current smoking, diabetes mellitus, hypertension, and body mass index), HDL and LDL cholesterol, and the use of statins at baseline and/or follow-up coronary CTA. Only lipid species with a Bonferroni-adjusted *P*-value <0.10 in the univariable analysis were included in the multivariable analysis. Lipid species were excluded from the current analysis if data were missing for >10 patients. All statistical analyses were performed using SPSS software package (IBM Corp Released 2017; IBM SPSS Statistics for Windows, Version 25.0; IBM Corp, Armonk, NY, USA).

Results

Baseline clinical characteristics

In total, 154 patients (66% male, mean age 62 ± 9 years) who underwent an interpretable serial coronary CTA and a plasma lipidomic analysis were included (*Table 1*). Seventy per cent of the patients had hypertension, 22% diabetes, and almost one-fifth of the patients were current smokers. Most patients (58%) underwent coronary CTA for atypical chest pain symptoms and 24% experienced typical chest pain. Over two-thirds of the patients received aspirin prior to their first coronary CTA. The great majority was administered statins between the first and the second CTA scans, 47% atorvastatin, 34% simvastatin, 13% rosuvastatin, and 6% pravastatin at standard dosage. Total, LDL, HDL, and triglyceride plasma levels were not significantly different between statin and non-statin users.

Coronary CTA data

Both total and calcified per-patient plaque volume significantly increased from baseline to follow-up coronary CTA (from 475 to 514 mm^3 and from 30 to 74 mm^3, respectively, both $P < 0.001$; *Table 2*). In contrast, non-calcified plaque volume modestly but significantly decreased from baseline to follow-up coronary CTA (from 420 to 417 mm^3, $P = 0.030$). Overall, both the number of plaques and the CAD score (reflecting the overall burden of CAD) increased from baseline to follow-up {from 4 [inter-quartile range (IQR) 1–6] to 5 [IQR 2–7] plaques and from 11 [IQR 5–19] to 14 [IQR 8–21], respectively, both $P < 0.001$}. While <30% was the maximal stenosis degree for most patients at the baseline coronary CTA, <30% and 30–50% were found to be equally prevalent as maximal stenosis degree at the follow-up coronary CTA.

Plasma lipid species vs. plaque changes according to composition

Plasma levels of lipid species encompassing six lipid classes and subclasses [cholesteryl ester (CE), ceramide (Cer), phosphatidylcholine (PC), phosphatidylserine (PS), sphingomyelin (SM), and triacylglycerol (TG)] were evaluated. A total of 9/55 (16%) lipid species were excluded from the current analysis, since data were missing for >10 patients. In the univariable regression analysis, no lipid species were significantly associated with annual total and calcified plaque changes according to the Bonferroni-adjusted *P*-values (*Tables 3* and *4*). In contrast, five lipid species were significantly associated with annual non-calcified plaque changes after the use of the Bonferroni correction method (*Table 5*). After adjusting for clinical variables at baseline, HDL and LDL cholesterol, and statin use at baseline and/or follow-up, three lipid species

Table 1 Baseline clinical characteristics

	Total (*N* = 154)	Non-calcified plaque progression (*N* = 90)	Non-calcified plaque regression (*N* = 64)	*P*-value
Age (years)	62 ± 9	62 ± 7	62 ± 12	0.94
Male	102 (66)	61 (68)	41 (64)	0.63
Current smoker	26 (17)	14 (16)	12 (19)	0.60
Diabetes	34 (22)	18 (20)	16 (25)	0.46
Hypertension	107 (70)	63 (70)	44 (69)	0.87
BMI	28 ± 4	28 ± 3	28 ± 4	0.55
Symptoms				
Typical	36 (24)	20 (23)	16 (26)	0.64
Atypical	87 (58)	53 (60)	34 (55)	0.56
Non-anginal	1 (1)	1 (1)	0 (0)	0.40
Other	20 (13)	9 (10)	11 (18)	0.17
No symptoms	7 (5)	6 (7)	1 (2)	0.14
Medication				
Beta-blockers	70 (46)	38 (42)	32 (50)	0.34
ACE-inhibitors/ARBs	79 (51)	51 (57)	28 (44)	0.11
Diuretics	28 (18)	17 (19)	11 (17)	0.79
Aspirin	105 (68)	60 (67)	45 (70)	0.63
Statin use	83 (54)	47 (52)	36 (56)	0.62
Total cholesterol level (mg/dL)	183 ± 50	190 ± 51	173 ± 46	**0.039**
LDL cholesterol level (mg/dL)	110 ± 42	116 ± 43	101 ± 38	**0.020**
HDL cholesterol level (mg/dL)	50 ± 15	50 ± 15	51 ± 14	0.80
Triglyceride level (mg/dL)	121 ± 59	127 ± 62	113 ± 56	0.15

Values are presented as mean ± SD or *n* (%). Values in bold font indicate statistically significant results.
ACE, angiotensin-converting enzyme; ARB, angiotensin-II receptor blocker; BMI, body mass index.

Table 2 Coronary CTA data

	Baseline (*N* = 154)	Follow-up (*N* = 154)	*P*-value
Total plaque volume (mm^3)	475 (173–902)	514 (190–1030)	**<0.001**
Calcified plaque volume (mm^3)	30 (10–70)	74 (21–150)	**<0.001**
Non-calcified plaque volume (mm^3)	420 (162–822)	417 (179–784)	**0.030**
Number of plaques	4 (1–6)	5 (2–7)	**<0.001**
CAD score[a]	11 (5–19)	14 (8–21)	**<0.001**
Maximal stenosis in all segments			
No CAD	14 (9)	0 (0)	N/A
<30%	59 (38)	53 (34)	0.34
30–50%	29 (19)	54 (35)	**<0.001**
50–70%	17 (11)	21 (14)	0.37
70–90%	26 (17)	17 (11)	**0.020**
>90%	8 (5)	9 (6)	0.71
Uninterpretable	1 (1)	0 (0)	N/A

Per-patient change in plaque characteristics between baseline and follow-up coronary CTA (*n* = 154). Values are presented as median (IQR) or *n* (%). Values in bold font indicate statistically significant results.
[a]Based on a comprehensive CTA score incorporating the presence, extent, severity, location, and composition of CAD.
N/A, not applicable.

Table 3 A linear regression analysis of the association between lipid biomarkers and annual total plaque changes

	Univariable[a]		
Lipid species	**Beta coefficient[b] (95% CI)**	**Unadjusted *P*-value**	**Bonferroni-adjusted *P*-value**
Cer(d18:1/16:0)	−1.39 (−4.00 to 1.23)	0.30	1.00
CE(14:0)	0.22 (−2.40 to 2.85)	0.87	1.00
CE(16:0)	−0.90 (−3.52 to 1.72)	0.50	1.00
CE(16:1)	0.89 (−1.73 to 3.52)	0.50	1.00
CE(18:0)	−0.21 (−2.84 to 2.41)	0.87	1.00
CE(18:1)	−0.69 (−3.31 to 1.93)	0.60	1.00
CE(18:2)	−0.97 (−3.59 to 1.65)	0.47	1.00
CE(18:3)	1.85 (−0.76 to 4.46)	0.16	1.00
CE(20:3)	3.52 (0.95–6.08)	**0.007**	0.34
CE(20:4)	3.79 (1.23–6.34)	**0.004**	0.18
CE(22:6)	3.28 (0.71–5.85)	**0.013**	0.59
PC(32:0)	1.14 (−1.48 to 3.76)	0.39	1.00
PC(32:1)	−0.39 (−3.02 to 2.23)	0.77	1.00
PC(34:3)	0.38 (−2.25 to 3.01)	0.78	1.00
PC(36:0)	0.69 (−1.93 to 3.31)	0.61	1.00
PC(36:1)	0.03 (−2.60 to 2.65)	0.99	1.00
PC(36:2)	−0.93 (−3.55 to 1.69)	0.48	1.00
PC(36:3)	2.41 (−0.19 to 5.01)	0.069	1.00
PC(36:4)	2.85 (0.27–5.44)	**0.031**	1.00
PC(38:2)	1.72 (−0.89 to 4.33)	0.19	1.00
PC(38:4)	2.98 (0.40–5.56)	**0.024**	1.00
PC(38:5)	2.85 (0.26–5.44)	**0.031**	1.00
PC(38:6)	3.17 (0.59–5.74)	**0.016**	0.75
PC(40:6)	2.81 (0.23–5.40)	**0.033**	1.00
PC(36:1e)/PC(36:0p)	0.35 (−2.28 to 2.97)	0.79	1.00
PC(36:2e)/PC(36:1p)	1.32 (−1.30 to 3.93)	0.32	1.00
TG(16:0/16:0/18:1)	0.76 (−1.87 to 3.38)	0.57	1.00
TG(16:0/16:1/18:1)	0.67 (−1.95 to 3.30)	0.61	1.00
TG(18:1/18:1/16:0)	0.34 (−2.29 to 2.96)	0.80	1.00
TG(18:1/18:1/16:1)	1.75 (−0.86 to 4.37)	0.19	1.00
TG(18:1/18:1/18:0)	1.81 (−0.80 to 4.42)	0.17	1.00
TG(18:1/18:1/18:1)	1.37 (−1.25 to 3.98)	0.30	1.00
PS(36:4)	0.64 (−1.99 to 3.26)	0.63	1.00
PS(38:5)	0.55 (−2.10 to 3.20)	0.68	1.00
PS(38:6)	1.98 (−0.72 to 4.67)	0.15	1.00
SM(36:2)	0.29 (−2.34 to 2.91)	0.83	1.00
SM(38:2)	0.90 (−1.73 to 3.52)	0.50	1.00
SM(38:1)	1.98 (−0.63 to 4.59)	0.14	1.00
SM(40:3)	1.40 (−1.06 to 3.86)	0.26	1.00
SM(40:2)	0.87 (−1.75 to 3.49)	0.51	1.00
SM(40:1)	−0.97 (−3.60 to 1.67)	0.47	1.00
SM(41:2)	0.87 (−1.75 to 3.49)	0.51	1.00
SM(41:1)	2.27 (−0.33 to 4.87)	0.087	1.00
SM(42:4)	1.60 (−1.01 to 4.22)	0.23	1.00
SM(42:3)	−0.20 (−2.84 to 2.43)	0.88	1.00
SM(42:1)	1.53 (−1.05 to 4.10)	0.24	1.00

Values in bold font indicate statistically significant results.
[a]No multivariable analysis was performed since all Bonferroni-adjusted *P*-values were >0.10 in the univariable analysis.
[b]Beta coefficients represent the change in annual total plaque volume for a 1-SD increase in the plasmatic concentration of lipid biomarker.
CI, confidence interval.

Table 4 A linear regression analysis of the association between lipid biomarkers and annual calcified plaque changes

	Univariable[a]		
Lipid species	**Beta coefficient[b] (95% CI)**	**Unadjusted *P*-value**	**Bonferroni-adjusted *P*-value**
Cer(d18:1/16:0)	−0.89 (−2.91 to 1.13)	0.39	1.00
CE(14:0)	−0.91 (−2.92 to 1.11)	0.38	1.00
CE(16:0)	0.06 (−1.96 to 2.08)	0.95	1.00
CE(16:1)	0.40 (−1.62 to 2.42)	0.70	1.00
CE(18:0)	−0.75 (−2.76 to 1.27)	0.47	1.00
CE(18:1)	−0.50 (−2.52 to 1.52)	0.62	1.00
CE(18:2)	−0.03 (−2.05 to 1.99)	0.98	1.00
CE(18:3)	−0.16 (−2.18 to 1.86)	0.88	1.00
CE(20:3)	0.26 (−1.76 to 2.28)	0.80	1.00
CE(20:4)	1.06 (−0.95 to 3.08)	0.30	1.00
CE(22:6)	1.17 (−0.85 to 3.18)	0.25	1.00
PC(32:0)	−0.18 (−2.20 to 1.84)	0.86	1.00
PC(32:1)	−0.33 (−2.35 to 1.70)	0.75	1.00
PC(34:3)	0.05 (−1.97 to 2.07)	0.96	1.00
PC(36:0)	−1.13 (−3.14 to 0.88)	0.27	1.00
PC(36:1)	−1.10 (−3.11 to 0.92)	0.28	1.00
PC(36:2)	−1.21 (−3.22 to 0.81)	0.24	1.00
PC(36:3)	−0.08 (−2.10 to 1.94)	0.94	1.00
PC(36:4)	1.03 (−0.98 to 3.05)	0.31	1.00
PC(38:2)	−0.40 (−2.42 to 1.62)	0.69	1.00
PC(38:4)	0.17 (−1.85 to 2.19)	0.87	1.00
PC(38:5)	0.61 (−1.41 to 2.63)	0.55	1.00
PC(38:6)	0.69 (−1.33 to 2.70)	0.50	1.00
PC(40:6)	0.31 (−1.71 to 2.33)	0.77	1.00
PC(36:1e)/PC(36:0p)	−0.75 (−2.76 to 1.27)	0.47	1.00
PC(36:2e)/PC(36:1p)	−0.80 (−2.81 to 1.22)	0.44	1.00
TG(16:0/16:0/18:1)	−0.50 (−2.52 to 1.52)	0.62	1.00
TG(16:0/16:1/18:1)	−0.36 (−2.38 to 1.66)	0.73	1.00
TG(18:1/18:1/16:0)	−1.58 (−3.58 to 0.43)	0.12	1.00
TG(18:1/18:1/16:1)	−0.33 (−2.35 to 1.69)	0.75	1.00
TG(18:1/18:1/18:0)	−0.92 (−2.94 to 1.09)	0.37	1.00
TG(18:1/18:1/18:1)	−0.82 (−2.83 to 1.20)	0.43	1.00
PS(36:4)	−0.70 (−2.72 to 1.32)	0.49	1.00
PS(38:5)	−0.77 (−2.73 to 1.18)	0.44	1.00
PS(38:6)	−0.21 (−1.80 to 2.22)	0.84	1.00
SM(36:2)	−1.22 (−3.23 to 0.79)	0.23	1.00
SM(38:2)	−0.86 (2.87–1.16)	0.40	1.00
SM(38:1)	−0.69 (−2.71 to 1.33)	0.50	1.00
SM(40:3)	−1.27 (−3.14 to 0.61)	0.18	1.00
SM(40:2)	−1.27 (−3.28 to 0.74)	0.21	1.00
SM(40:1)	−1.23 (−3.25 to 0.78)	0.23	1.00
SM(41:2)	−1.42 (−3.43 to 0.58)	0.16	1.00
SM(41:1)	−1.49 (−3.49 to 0.52)	0.15	1.00
SM(42:4)	−0.69 (−2.71 to 1.33)	0.50	1.00
SM(42:3)	−1.07 (−3.10 to 0.96)	0.30	1.00
SM(42:1)	−0.90 (−2.93 to 1.13)	0.38	1.00

[a]No multivariable analysis was performed since all Bonferroni-adjusted *P*-values were >0.10 in the univariable analysis.
[b]Beta coefficients represent the change in annual calcified plaque volume for a 1-SD increase in the plasmatic concentration of lipid biomarker.
CI, confidence interval.

Table 5 A linear regression analysis of the association between lipid biomarkers and annual non-calcified plaque changes

	Univariable			Multivariable[a]		
Lipid species	**Beta coefficient[b] (95% CI)**	**Unadjusted *P*-value**	**Bonferroni-adjusted *P*-value**	**Beta coefficient[b] (95% CI)**	**Unadjusted *P*-value**	**Bonferroni-adjusted *P*-value**
Cer(d18:1/16:0)	−0.23 (−2.68 to 2.21)	0.85	1.00			
CE(14:0)	2.37 (−0.05 to 4.79)	0.054	1.00			
CE(16:0)	−1.94 (−4.37 to 0.49)	0.12	1.00			
CE(16:1)	0.61 (−1.83 to 3.06)	0.62	1.00			
CE(18:0)	1.01 (−1.43 to 3.46)	0.41	1.00			
CE(18:1)	−1.20 (−3.64 to 1.24)	0.33	1.00			
CE(18:2)	−2.32 (−4.74 to 0.10)	0.060	1.00			
CE(18:3)	3.41 (1.03–5.80)	**0.005**	0.25			
CE(20:3)	4.75 (2.43–7.08)	**<0.001**	**0.004**	5.06 (2.55–7.58)	**<0.001**	**0.005**
CE(20:4)	3.47 (1.09–5.86)	**0.005**	0.21			
CE(22:6)	2.84 (0.44–5.25)	**0.021**	0.96			
PC(32:0)	2.29 (−0.13 to 4.71)	0.063	1.00			
PC(32:1)	0.52 (−1.93 to 2.96)	0.68	1.00			
PC(34:3)	1.02 (−1.42 to 3.46)	0.41	1.00			
PC(36:0)	2.76 (0.35–5.16)	**0.025**	1.00			
PC(36:1)	1.28 (−1.16 to 3.72)	0.30	1.00			
PC(36:2)	−0.37 (−2.82 to 2.08)	0.77	1.00			
PC(36:3)	3.05 (0.66–5.45)	**0.013**	0.59			
PC(36:4)	2.38 (−0.04 to 4.79)	0.054	1.00			
PC(38:2)	2.68 (0.27–5.09)	**0.030**	1.00			
PC(38:4)	3.63 (1.25–6.01)	**0.003**	0.14			
PC(38:5)	3.32 (0.93–5.71)	**0.007**	0.31			
PC(38:6)	3.24 (0.85–5.63)	**0.008**	0.38			
PC(40:6)	3.48 (1.10–5.87)	**0.004**	0.20			
PC(36:1e)/PC(36:0p)	1.87 (−0.56 to 4.30)	0.13	1.00			
PC(36:2e)/PC(36:1p)	3.16 (0.76–5.55)	**0.010**	0.46			
TG(16:0/16:0/18:1)	2.81 (0.40–5.21)	**0.022**	1.00			
TG(16:0/16:1/18:1)	2.46 (0.05–4.88)	**0.046**	1.00			
TG(18:1/18:1/16:0)	1.79 (−0.64 to 4.22)	0.15	1.00			
TG(18:1/18:1/16:1)	2.37 (−0.05 to 4.78)	0.055	1.00			
TG(18:1/18:1/18:0)	3.35 (0.96–5.74)	**0.006**	0.29			
TG(18:1/18:1/18:1)	1.80 (−0.63 to 4.23)	0.15	1.00			
PS(36:4)	1.90 (−0.53 to 4.33)	0.13	1.00			
PS(38:5)	2.41 (0.13–4.68)	**0.039**	1.00			
PS(38:6)	2.36 (0.18–4.54)	**0.034**	1.00			

Continued

Table 5 Continued

Lipid species	Univariable			Multivariable[a]		
	Beta coefficient[b] (95% CI)	Unadjusted *P*-value	Bonferroni-adjusted *P*-value	Beta coefficient[b] (95% CI)	Unadjusted *P*-value	Bonferroni-adjusted *P*-value
SM(36:2)	2.37 (−0.05 to 4.79)	0.055	1.00			
SM(38:2)	2.82 (0.41–5.23)	**0.022**	1.00			
SM(38:1)	4.29 (1.94–6.64)	**<0.001**	**0.019**	4.05 (1.28–6.83)	**0.005**	0.21
SM(40:3)	4.28 (1.86–6.70)	**0.001**	**0.029**	4.98 (2.30–7.66)	**<0.001**	**0.016**
SM(40:2)	3.59 (1.21–5.97)	**0.003**	0.15			
SM(40:1)	1.65 (−0.78 to 4.07)	0.18	1.00			
SM(41:2)	3.68 (1.30–6.05)	**0.003**	0.12			
SM(41:1)	5.29 (2.99–7.59)	**<0.001**	**<0.001**	6.23 (3.18–9.27)	**<0.001**	**0.004**
SM(42:4)	3.89 (1.53–6.26)	**0.001**	0.065	4.43 (1.58–7.28)	**0.003**	0.12
SM(42:3)	1.38 (−1.07 to 3.82)	0.27	1.00			
SM(42:1)	4.06 (1.72–6.40)	**0.001**	**0.037**	4.30 (1.07–7.54)	**0.010**	0.44

Values in bold font indicate statistically significant results.
[a]A multivariable analysis was performed adjusting for cardiovascular risk factors (i.e. age, gender, current smoking, diabetes mellitus, hypertension, body mass index, HDL, and LDL) and the use of statins at baseline and/or follow-up coronary CTA. Only lipid species with a Bonferroni-adjusted *P*-value <0.10 in the univariable analysis were included in the multivariable analysis.
[b]Beta coefficients represent the change in annual non-calcified plaque volume for a 1-SD increase in the plasmatic concentration of lipid biomarker.
CI, confidence interval.

remained significantly associated with non-calcified plaque changes. In detail, CE(20:3), SM(40:3), and SM(41:1) were found positively related to non-calcified plaque progression (Bonferroni-adjusted *P*-values = 0.005, 0.016, and 0.004, respectively). A 1-SD increase in the plasmatic concentration of these lipid biomarkers led to an annual non-calcified plaque volume increase of 5.1, 5.0, and 6.2 mm^3, respectively.

Discussion

The relationship between lipid species by a plasma lipidomic analysis and coronary plaque changes assessed by a quantitative CTA was prospectively evaluated. We found that three of the analysed lipid species were significantly associated with non-calcified plaque progression, independent of clinical risk factors, HDL and LDL cholesterol, and statin therapy. In contrast, no lipid species were significantly associated with total and calcified plaque changes.

Non-calcified plaque vs. cardiovascular outcome

The finding that specific lipid species were associated with non-calcified plaque progression could have important clinical relevance, since prior studies have shown that non-calcified plaques are at a higher risk of rupture and thereby potentially triggering an acute coronary syndrome. van Werkhoven *et al.*[5] studied the incremental prognostic value of coronary CTA characteristics over coronary artery calcium scoring in 432 patients with suspected CAD. During a median follow-up of almost 2 years, the authors demonstrated that the number of segments with non-calcified and mixed plaques, but not with calcified plaques, was significantly associated with adverse cardiac outcomes. These findings were in agreement with a study performed by Hou *et al.*,[6] in which 5007 patients who underwent coronary CTA for suspected CAD were included. During a median follow-up of 3 years, it was shown that non-calcified and mixed plaques were strongly associated with cardiac death and myocardial infarction. Moreover, Motoyama *et al.*[7,8] studied atherosclerotic lesions in 3158 patients who underwent coronary CTA during a mean follow-up of 3.9 years. It was found that the presence of high-risk plaques, defined as plaques with low-attenuation non-calcified plaque and/or positive remodelling, was an independent predictor of acute coronary syndrome.

Cross-association between lipid species vs. non-calcified plaque and cardiovascular outcome

To our knowledge, our study is the first to determine the association between lipid species and CAD progression using serial imaging. Nevertheless, several studies have been performed on the cross-association of lipid species with present CAD (i.e. the assessment of coronary plaque characteristics on a single computed tomography scan or other imaging modality) and/or cardiovascular outcome. Ellims *et al.*[3] prospectively performed coronary CTA and plasma lipid profiling in 100 asymptomatic patients at intermediate risk of CAD. A visual analysis of coronary CTA scans was performed to evaluate the burden of total, calcified, and non-calcified plaques by the use of the segment stenosis score. Interestingly, the authors found that 18 lipid species were significantly associated with non-calcified plaque burden, but not with the burden of total or calcified plaque. In addition, Cheng *et al.*[4] analysed 581 patients who underwent coronary angiography for acute coronary syndrome or stable angina. In all patients, intravascular ultrasound virtual histology imaging of a non-culprit coronary artery was performed to assess a necrotic core fraction. Moreover, near-infrared spectroscopy was performed in 191 patients to assess the lipid core burden index. Several cholesteryl, ceramide, and lactosylceramide lipid species were shown to be associated with a higher necrotic core fraction and lipid core burden index. Moreover, a significant association between several lipid species and 1-year cardiovascular outcome was noted. Anroedh *et al.*[10] investigated the association of 10 lipid species and 3 ceramide ratios with cardiovascular outcomes during a median follow-up of 4.7 years. The authors

demonstrated that several ceramide lipid species were significantly associated with adverse cardiac outcomes, independent of risk factor profile, statin therapy, and cholesterol level. Also, Tarasov *et al.*[11] showed that several ceramide lipid species were significantly associated with cardiovascular death in 445 CAD patients with a long-term follow-up. Interestingly, Meikle *et al.*[18] studied the ability of a plasma lipidomic analysis to discriminate patients with unstable CAD from stable CAD. For this purpose, 305 lipids were measured in 220 individuals (of whom 80 were controls, 60 with stable CAD, and 80 with unstable CAD). The authors found that models using a combination of both lipids and traditional risk factors performed significantly better in their ability to discern between stable vs. unstable CAD patients, compared with a model with only risk factors. Accordingly, plasma lipid profiling could not only be of clinical value to predict non-calcified plaque progression and clinical outcome but also to identify patients with unstable CAD.

Lipid species vs. progression of non-calcified plaque

In the present study, we found a significant relationship between four of the analysed lipid species and non-calcified plaque progression, which was independent of clinical risk profile and statin therapy.

Vulnerable non-calcified plaques are predominantly characterized by extracellular lipid accumulations also known as lipid cores. These lipid cores dramatically increase intimal wall shear stress compared with fibrous tissues and calcifications, rendering lipid-rich atherosclerotic plaques far more unstable and vulnerable to sudden rupture than other types of lesions.[19] Lipid species here related to non-calcified plaque progression mainly belong to SM, PC, CE, and TG classes. These lipids could affect the progression of non-calcified plaques in different and opposite ways. On one side, CEs and oxidized phospholipids, mainly aggregated in the form of LDL, can cross the endothelial barrier and enter the subendothelial space of the arterial wall, thus contributing to the development of atheroma. On the other hand, SMs and PCs are reported to be the most abundant lipid components in HDL particles and, according to their surface distribution, play a key role in cholesterol efflux capacity.[20] Dysfunctional HDL exhibit altered compositions in contents of SM and PC and a substitution of 50% of CE for TG.[21] These lipid changes can alter anti-atherogenic HDL assets, reducing their cholesterol efflux capacity from peripheral cells and hindering reverse cholesterol transport, thus limiting the possibility of preventing atherosclerosis.

However, lipid retention is only the first step in the pathogenesis of atherosclerosis, which is followed by chronic inflammation at susceptible sites in the walls of the major arteries leading to non-calcified atheromas, which then progress to fibroatheromas with fibrous thickening and calcium deposition. Calcified plaques represent a late stage in coronary atherosclerosis during which dynamic interactions with plasma lipids have likely diminished.[3]

The lipid species associated with non-calcified plaque progression could be used in addition to clinical parameters that are known to be predictive of CAD progression. As shown in our SMARTool main clinical study, hypertension was an independent determinant of non-calcified plaque progression.[9] Accordingly, patients with hypertension in combination with a high plasma concentration of certain lipid species could have a higher risk of non-calcified plaque progression. Intensified preventive treatment by lifestyle interventions and/or statin therapy may be specifically needed in these patients to slow or prevent CAD progression and improve cardiovascular outcome.

Limitations

Our study has certain limitations. First, several morphological plaque characteristics that are known to be markers of high-risk plaque (e.g. low-attenuation plaque, positive remodelling, napkin-ring sign, and spotty calcification) were not included in the current analysis. Second, 16% of the quantified lipid species had to be excluded from the analysis since data were missing for >10 patients at the baseline coronary CTA. Finally, the limitations previously described for the SMARTool main clinical study also apply to the current study.[9] These include the use of coronary CTA scanners from different vendors, the lack of information on possible changes in treatment in the inter-scan period, and the unavailability of high-risk plaque features (e.g. napkin-ring sign and spotty calcification).

Conclusion

The current study showed an independent relationship between specific lipid species determined by a plasma lipidomic analysis and non-calcified coronary plaque progression assessed by a serial, quantitative coronary CTA analysis. Future studies are needed to further investigate the relationship among plasma lipid species, coronary plaque progression, and cardiovascular outcome.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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