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Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: Results of the ATHEROREMO-IVUS study

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ABSTRACT

Background and Aims: Previous lipidomics analyses have demonstrated that several lipid molecules in plasma are associated with fatal outcome in patients with coronary artery disease (CAD). This study aims to investigate the associations of previously identified high risk lipid molecules in plasma with coronary plaque characteristics derived from intravascular ultrasound virtual histology (IVUS-VH) imaging, with coronary lipid core burden index (LCBI) on near-infrared spectroscopy (NIRS), and with one year cardiovascular outcome in patients with CAD.

Methods: Between 2008 and 2011, IVUS-VH imaging of a non-culprit coronary artery was performed in 581 patients who underwent coronary angiography for acute coronary syndrome (ACS) or stable CAD. NIRS imaging was additionally performed in 191 patients. Plasma concentrations of molecular lipids were measured with mass spectrometry.

Results: Several cholesteryl ester, ceramide and lactosylceramide species and ceramide ratios were associated with vulnerable plaque characteristics on IVUS-VH and NIRS imaging and with 1-year major adverse cardiac events (MACE, defined as all-cause mortality, ACS and unplanned coronary revascularization). In particular, ceramide d18:1/16:0 was consistently associated with higher necrotic core fraction on IVUS-VH ($p = 0.001$), higher LCBI ($p = 0.024$) on NIRS and higher MACE rate (adjusted HR 1.79 per standard deviation increase in log-transformed lipid concentration, 95%CI 1.24–2.59, $p = 0.002$).

Conclusion: Several molecular lipid species, and particularly ceramide(d18:1/16:0), are associated with the fraction of necrotic core tissue and lipid core burden in coronary atherosclerosis, and are predictive for 1-year clinical outcome after coronary angiography. These molecular lipids may improve risk stratification in CAD and may also be interesting therapeutic targets for the treatment of atherosclerotic disease.

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1. Introduction

In current clinical practice, the concentration of low-density lipoprotein (LDL) cholesterol is often used for risk stratification in coronary artery disease (CAD). However, LDL cholesterol represents merely one aspect of lipid metabolism. Lipidomic analyses have demonstrated that hundreds of molecular lipid species are present in human plasma [1]. It is reasonable to assume that some of these molecular lipid species are also directly involved in the development of atherosclerosis [2–4]. Assessment of such 'high risk' molecular lipids may further improve our understanding of the development of atherosclerosis and may also improve CAD risk stratification. In fact, we have recently identified several molecular lipid species that are associated with fatal outcome in patients with CAD by performing lipidomic analysis in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study [5].

So far, lipidomics studies in CAD have mostly examined associations with clinical cardiovascular outcomes. Investigations using sophisticated imaging techniques may provide further insight into the pathophysiological role of lipid species in CAD. Intravascular ultrasound virtual histology (IVUS-VH) is an in-vivo imaging technique that analyzes radiofrequency backscatter [6]. IVUS-VH imaging allows for accurate measurement of the extent of coronary atherosclerosis and of the composition of atherosclerotic plaque, including necrotic core tissue [6–9]. Previous studies have demonstrated that the amount of necrotic core tissue on IVUS-VH predicts cardiovascular outcome [7–9]. Near-infrared spectroscopy (NIRS) is another in-vivo imaging technique that analyzes tissue scattering and absorption of light in the near-infrared wavelength region. NIRS allows for identification of plaques with lipid cores in coronary atherosclerosis [10]. We have recently demonstrated that the lipid core burden assessed by NIRS predicts cardiovascular outcome [11].

This study aims to investigate the associations of high risk molecular lipids, previously identified in the LURIC study, with coronary plaque characteristics assessed by IVUS-VH imaging, with coronary lipid core burden assessed by NIRS imaging, and with 1 year cardiovascular outcome in patients with CAD.

2. Methods

2.1. Study population

The design of The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis – Intravascular Ultrasound (ATHEROREMO-IVUS) study has been described in detail elsewhere [7,12]. In brief, 581 patients who underwent diagnostic coronary angiography or percutaneous coronary intervention (PCI) for acute coronary syndrome (ACS) or stable angina pectoris were included between 2008 and 2011 in the Erasmus MC, Rotterdam, the Netherlands. The ATHEROREMO-IVUS study was approved by the medical ethics committee of the Erasmus MC. The study was performed in accordance with the criteria described in the declaration of Helsinki. Written informed consent was obtained from all included patients. This study is registered in ClinicalTrials.gov, number NCT01789411.

2.2. Plasma concentrations of molecular lipids

Blood samples were drawn prior to coronary angiography, and were stored at a temperature of −80 °C. Stored plasma samples (n = 574 patients) were subjected to lipid extraction [13]. The extracts were reconstituted as described elsewhere [13]. Sphingolipids were analyzed on a QTRAP® 5500 mass spectrometer (AB SCIEX, Concord, Canada) equipped with an ultra-high pressure liquid chromatography (UHPLC) system; CTC PAL autosampler (Leap Technologies) and Rheos Allegro UHPLC (Flux Instruments) using multiple reaction monitoring in positive ion mode [14], using an acquity BEH C18, 2.1 × 50 mm column with a particle size of 1.7 μm (Waters, Milford, MA). A 25 min gradient using 10 mM ammonium acetate in water with 0.1% formic acid (mobile phase A) and 10 mM ammonium acetate in acetonitrile:isopropanol (4:3, v/v) containing 0.1% formic acid (mobile phase B) was applied. Shotgun lipidomics was performed by multiple precursor ion and neutral loss scanning on a QTRAP® 5500 mass spectrometer (AB SCIEX, Concord, Canada) equipped with a robotic nanoflow ion source NanoMate HD (Advion, NY, USA) [15]. Mass spectrometry data files were processed using MultiQuant™ 1.1.0.26 or Lipid Profiler™ 1.1 (AB SCIEX, Concord, Canada) [16]. Quality control samples are utilized to monitor the overall quality of the lipid extraction and mass spectrometry analyses [17]. In 7 patients, plasma samples were not available for measurement of molecular lipid concentrations.

Molecular lipids and lipid ratios that were previously found to be associated with fatal cardiovascular outcome at a $p < 0.001$ level in the Ludwigshafen Risk and Cardiovascular Health (LURIC) lipidomic study were selected for evaluation in this study [5]. These include 8 molecular lipids [cholesteryl ester (CE) 14:0, CE 18:3, CE 20:4, CE 20:5, CE 22:5, ceramide (Cer) (d18:1/16:0), Cer(d18:1/24:0), lactosylceramide (LacCer) (d18:1/18:0)] and 3 ceramide ratios [Cer(d18:1/16:0)/Cer(d18:1/24:0), Cer(d18:1/20:0)/Cer(d18:1/24:0), Cer(d18:1/24:1)/Cer(d18:1/24:0)].

2.3. Coronary intravascular ultrasound imaging

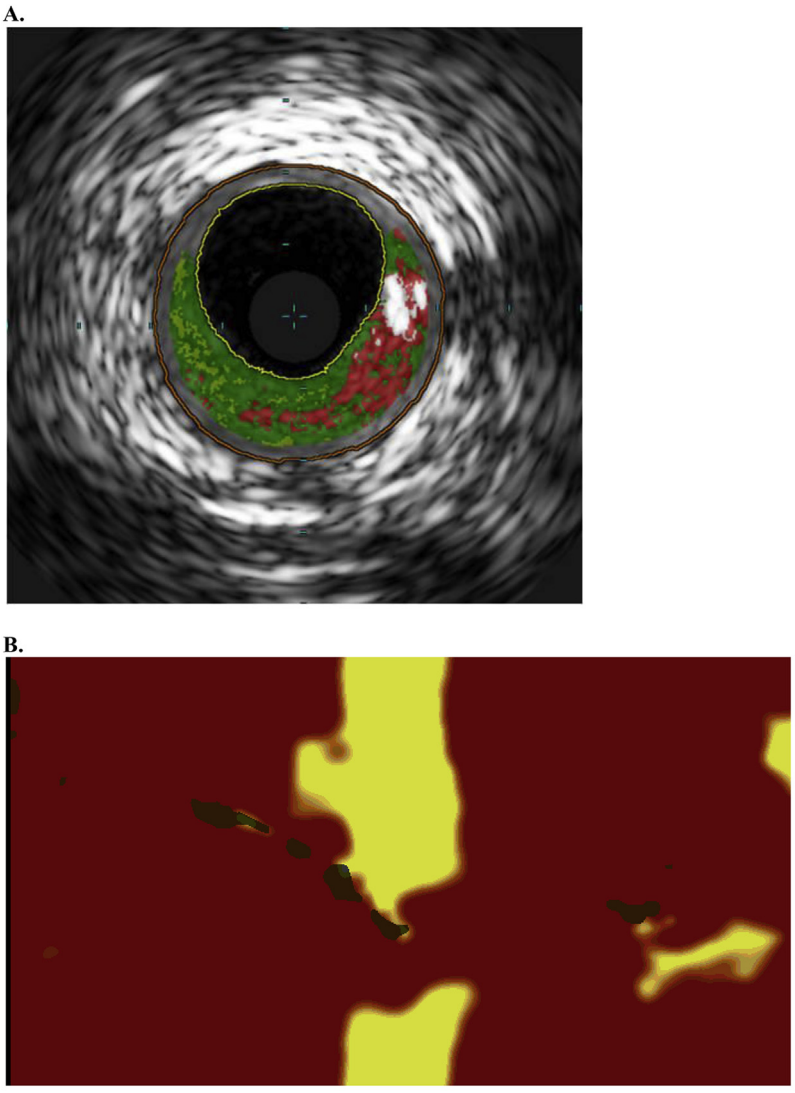
Following the standard coronary angiography procedure, IVUS-VH imaging of the most proximal part of a non-culprit coronary artery was performed. All IVUS-VH data were acquired with the Volcano s5/s5i Imaging System (Volcano Corp., San Diego, CA, USA) using a Volcano Eagle Eye Gold IVUS-VH catheter (20 MHz). The IVUS-VH images were sent to an independent core laboratory (Cardialysis bv, Rotterdam, the Netherlands) for offline analysis. The core laboratory personnel were blinded for patient characteristics, molecular lipids and clinical outcome data. Extent and phenotype of the atherosclerotic plaque were assessed. Plaque burden was defined as the plaque and media cross-sectional area divided by the external elastic membrane cross-sectional area. The composition of atherosclerotic plaque was characterized into 4 different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core (Fig. 1) [6].

2.4. Coronary near-infrared spectroscopy imaging

A total of 191 patients also provided written informed consent for additional enrollment in the ATHEROREMO-NIRS substudy, in which NIRS imaging of the non-culprit coronary artery was additionally performed [12]. All NIRS data were acquired with the InfraReDx NIRS system (InfraReDx, Burlington, Massachusetts, USA) [18,19]. Areas of the artery with spectral characteristics of lipid core are displayed as an image map (chemogram) of the studied vessel. The lipid core burden index (LCBI) score is computed on the basis of the chemogram by multiplying the fraction of valid yellow pixels by 1.000 (Fig. 1). NIRS images were analyzed offline by an independent core laboratory (Cardialysis BV, Rotterdam, The Netherlands).

2.5. Follow-up and clinical endpoints

Clinical follow-up started at inclusion and lasted for 1 year. Post-discharge survival status was obtained from municipal civil



A. Intravascular ultrasound virtual histology imaging was used to characterize atherosclerotic plaque into 4 different tissue types: fibrous (dark green), fibro-fatty (light green), dense calcium (white) and necrotic core (red). B. Near-infrared spectroscopy was used to compute the lipid core burden index (LCBI) score on basis of the chemogram by multiplying the fraction of valid yellow pixels within the region of interest by 1000.

Fig. 1. Coronary intravascular ultrasound virtual histology imaging and near-infrared spectroscopy.

registries. Questionnaires focusing on the occurrence of major adverse cardiac events (MACE) were sent to all living patients. Subsequently, hospital discharge letters were obtained and treating physicians and institutions were contacted for additional information (i.e. discharge letters and coronary angiogram) whenever necessary. All events were adjudicated either as being related to the coronary site that was treated during the index procedure (culprit lesion-related event) or as being related to a coronary site that was not treated during the index procedure (non-culprit lesion-related event). Events that were related to both the culprit lesion and a non-culprit site (e.g. revascularization of multiple vessels) were classified into both categories. When information was not sufficient to classify an event as either culprit lesion related or non-culprit lesion related, the event was classified as indeterminate.

Occurrence of culprit lesion-related events is most likely caused by in-stent restenosis or in-stent thrombosis. Since, in the current study, we were specifically interested in unanticipated, spontaneous MACE, we took into account only those clinical endpoints that were defined as non-culprit lesion-related or indeterminate. These clinical endpoints were: 1. MACE, defined as non-culprit lesion-related or indeterminate mortality, ACS or unplanned coronary revascularization; and 2. the composite of non-culprit lesion-related or indeterminate mortality or ACS, both during 1 year of follow-up. ACS was defined as the clinical diagnosis of ST segment elevation myocardial infarction, non-ST segment elevation myocardial infarction or unstable angina pectoris. Unplanned coronary revascularization was defined as unplanned repeat PCI or coronary artery bypass grafting (CABG). The endpoints were adjudicated by a clinical event committee that had no knowledge of the molecular lipids and the IVUS-VH data.

2.6. Statistical analysis

Prior to statistical analysis the molecular lipids were log-transformed. Unpaired Student's t-test was used to evaluate the difference in mean log-transformed lipid concentration between

the highest quartile and the lowest quartile of 1. plaque burden; 2. fibrous tissue percentage; 3. fibrofatty tissue percentage; 4. dense calcium percentage; 5. necrotic core percentage; and 6. LCBI. Patients lost during the follow-up were considered at risk until the date of last contact, at which time-point they were censored. Cumulative event rates were estimated according to the Kaplan–Meier method. Cox proportional hazards regression analyses were performed to evaluate the associations between molecular lipids and clinical study endpoints. Age, gender, type 2 diabetes, statin use at time of hospital admission and clinical presentation (ACS or stable CAD) were a priori defined as potential confounders, and were therefore entered as covariates in multivariate analyses. Also, baseline serum LDL cholesterol level was additionally entered into the model to evaluate whether the associations with MACE were independent of serum LDL cholesterol level. Unadjusted and adjusted hazard ratios (HR) with 95% CI were reported.

All data were analyzed with SPSS software (SPSS 20.0, IBM corp., Armonk, NY, USA). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant. Since the lipids of interest were selected a priori, neither false discovery rates were estimated nor corrections for multiple hypotheses testing were applied.

3. Results

3.1. Baseline characteristics

Mean age of the patients was 61.5 ± 11.3 years, 75% were men, and 55% were admitted with ACS (Table 1). ACS patients had higher concentrations of CE 14:0, CE 18:3, CE 22:5, Cer(d18:1/16:0), Cer(d18:1/24:0), LacCer(d18:1/18:0) and Cer(d18:1/16:0)/Cer(d18:1/24:0) than patients with stable CAD (Table 2). Patients who used statins at hospital admission had lower concentrations of the majority of the molecular lipids compared to patients who did not used statins (Supplemental Table 1).

3.2. Association between molecular lipids and coronary plaque characteristics

Patients whose necrotic core fraction was in the highest quartile had higher concentrations of CE 18:3 (80.5 vs. 72.7 pmol/μl, p = 0.040), CE 20:5 (62.8 vs. 52.4 pmol/μl, p = 0.022), Cer(d18:1/16:0) (0.139 vs. 0.122 pmol/μl, p = 0.001) and Cer(d18:1/24:0) (6.94 vs. 6.05 pmol/μl, p = 0.003) compared to patients whose necrotic core fraction was in the lowest quartile (Table 3, Supplemental Table 2). Patients whose LCBI was in the highest quartile had higher concentrations of Cer(d18:1/16:0) (0.125 vs. 0.112 pmol/μl, p = 0.024), Cer(d18:1/24:0) (6.76 vs. 5.42 pmol/μl, p = 0.001) and LacCer(d18:1/18:0) (0.134 vs. 0.119 pmol/μl, p = 0.049) compared to patients whose LCBI was in the lowest quartile. The Cer(d18:1/20:0)/Cer(d18:1/24:0) ratio was higher in patients in the highest plaque burden quartile compared to the lowest quartile (0.0215 vs. 0.0194, p = 0.036), as well as in patients in the highest dense calcium quartile compared to the lowest quartile (0.0214 vs. 0.0193, p = 0.009). There was no association between the presence of IVUS-VH-derived thin-cap fibroatheroma lesions and the molecular lipid concentrations (Supplemental Table 3).

3.3. Incident major adverse cardiac events

Vital status at 1-year follow-up could be acquired for 572 (99.7%) patients. Response rate of the questionnaires that were sent to all living patients was 92.0%. After 1 year of follow-up, 56 patients had experienced a MACE (Supplemental Table 4). The cumulative Kaplan–Meier incidences of the 30-day, 6-month and 1-year MACE (definite culprit lesion-related events not included as endpoint) were 0.7%, 4.7%, and 7.9%, respectively. The cumulative Kaplan–Meier incidences of the 30-day, 6-month and 1-year composite of death or ACS (definite culprit lesion-related events not included) were 0.7%, 3.1%, and 4.9%, respectively.

Table 1
Baseline characteristics.

	n = 574 patients
Patient characteristics	
Age, years	61.5 ± 11.3
Men, n (%)	432 (75.3)
Diabetes Mellitus, n (%)	97 (16.9)
Hypertension, n (%)	298 (51.9)
Hypercholesterolemia, n (%)	318 (55.4)
Current smoking, n (%)	166 (28.9)
Positive family history, n (%)	298 (51.9)
Previous MI, n (%)	184 (32.1)
Previous PCI, n (%)	184 (32.1)
Previous CABG, n (%)	18 (3.1)
Previous stroke, n (%)	26 (4.5)
History of peripheral artery disease, n (%)	35 (6.1)
History of renal insufficiency, n (%)	32 (5.6)
History of heart failure, n (%)	19 (3.3)
Serum LDL cholesterol, mmol/L	2.71 [2.12–3.54]
Statin use at time of hospital admission, n (%)	357 (62.2)
Procedural characteristics	
Indication for coronary angiography	
ACS, n (%)	313 (54.5)
ST-elevation MI	162 (28.2)
Non-ST-elevation ACS	151 (26.3)
Stable coronary artery disease, n (%)	261 (45.5)
Number of diseased coronary vessels[a]	
No significant stenosis, n (%)	42 (7.3)
1-vessel disease, n (%)	304 (53.0)
2-vessel disease, n (%)	167 (29.1)
3-vessel disease, n (%)	61 (10.6)
PCI performed, n (%)	505 (88.0)
IVUS-VH imaging	
Imaged coronary artery	
Left anterior descending, n (%)	206 (35.9)
Left circumflex, n (%)	192 (33.4)
Right coronary artery, n (%)	176 (30.7)
Segment length, mm	44.1 [33.7–55.4]
Plaque burden, %	38.2 ± 11.5
Fibrous tissue fraction, %	57.8 ± 11.6
Fibro-fatty tissue fraction, %	9.91 ± 6.3
Dense calcium fraction, %	10.9 ± 7.7
Necrotic core fraction, %	21.4 ± 8.1
NIRS imaging[b]	
Lipid core burden index	43 [15–83]

Data are presented as mean ± standard deviation or as median [interquartile range].
ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; IVUS-VH, intravascular ultrasound virtual histology; LDL, low-density lipoprotein; MI, myocardial infarction; NIRS, near-infrared spectroscopy; PCI, percutaneous coronary intervention.
[a] A significant stenosis was defined as a stenosis ≥50% of vessel diameter by visual assessment on the coronary angiogram.
[b] NIRS imaging was performed in 191 patients in addition to IVUS-VH imaging.

3.4. Association between molecular lipids and cardiovascular outcome

In univariate analysis, Cer(d18:1/16:0) concentration was associated with 1-year incidence of MACE (HR 1.44 per standard deviation increase in log-transformed lipid concentration, 95%CI 1.08–1.93, p = 0.014) and the composite endpoint of death or ACS (HR 1.86, 95%CI 1.29–2.69, p = 0.001), as well as with the individual components of death (borderline significant, HR 1.53, 95%CI 0.97–2.42, p = 0.068) and ACS (HR 2.36, 95%CI 1.32–4.21, p = 0.004) (Supplemental Table 5) (Fig. 2). LacCer(d18:1/18:0) and

Table 2
Lipid concentrations.

	Total (n = 574)	ACS (n = 313)	Stable CAD (n = 261)	P
CE 14:0, pmol/μl	21.7 [15.9–28.1]	22.9 [16.5–30.5]	21.2 [15.4–26.8]	0.008
CE 18:3, pmol/μl	70.3 [51.8–90.7]	72.3 [53.6–99.5]	66.1 [50.3–85.2]	0.003
CE 20:4, pmol/μl	386 [317–457]	394 [324–453]	374 [307–471]	0.31
CE 20:5, pmol/μl	49.1 [36.3–72.6]	49.2 [36.4–72.1]	49.0 [35.9–74.7]	0.69
CE 22:5, pmol/μl	2.65 [2.00–3.62]	2.81 [2.12–3.77]	2.53 [1.90–3.40]	0.037
Cer(d18:1/16:0), pmol/μl	0.12 [0.10–0.15]	0.13 [0.11–0.17]	0.11 [0.09–0.13]	<0.001
Cer(d18:1/24:0), pmol/μl	5.98 [4.72–7.49]	6.43 [5.00–8.07]	5.65 [4.49–6.61]	<0.001
LacCer(d18:1/18:0), pmol/μl	0.13 [0.10–0.16]	0.13 [0.11–0.16]	0.12 [0.10–0.15]	0.001
Cer(d18:1/16:0)/Cer(d18:1/24:0)	0.020 [0.018–0.024]	0.021 [0.018–0.025]	0.020 [0.017–0.023]	0.001
Cer(d18:1/20:0)/Cer(d18:1/24:0)	0.019 [0.016–0.024]	0.019 [0.015–0.024]	0.019 [0.016–0.023]	0.62
Cer(d18:1/24:1)/Cer(d18:1/24:0)	0.31 [0.26–0.36]	0.31 [0.26–0.36]	0.31 [0.26–0.36]	0.65

Concentrations are presented in μM. Data are presented as median [interquartile range]. P-value was obtained from Student's t-test for difference in log-transformed mean lipid concentration.
ACS, acute coronary syndrome; CAD, coronary artery disease.

the ratios of Cer(d18:1/16:0)/Cer(d18:1/24:0), Cer(d18:1/20:0)/Cer(d18:1/24:0) and Cer(d18:1/24:1)/Cer(d18:1/24:0) were also associated with the composite endpoint of death or ACS, but were only driven by death.

After adjustment for patient characteristics, statin use and clinical presentation in multivariate analysis, Cer(d18:1/16:0) remained significantly associated with MACE (HR 1.61, 95%CI 1.17–2.22, p = 0.004) and the composite endpoint of death or ACS (HR 1.88, 95%CI 1.24–2.84, p = 0.003) (Table 4, Supplemental Table 6). After additional adjustment for admission LDL cholesterol level, Cer(d18:1/16:0) also remained significantly associated with MACE (HR 1.79, 95%CI 1.24–2.59, p = 0.002) and the composite endpoint of death or ACS (HR 2.45, 95%CI 1.55–3.87, p < 0.001).

4. Discussion

This study investigated the association of eight previously identified high risk cholesteryl ester, ceramide and lactosylceramide lipids and three ceramide ratios with coronary plaque characteristics on IVUS-VH and NIRS imaging, as well as with 1-year clinical outcome in patients with established CAD undergoing coronary angiography. The main finding is that higher plasma concentrations of several of these molecular lipid species are associated with more vulnerable plaque morphology, reflected by a higher fraction of coronary plaque consisting of necrotic core tissue on IVUS-VH and by a higher lipid core burden on NIRS imaging. This is the first study that has demonstrated such an association for molecular lipid species circulating in plasma. Secondly, Cer(d18:1/16:0) predicts 1-year MACE after coronary angiography, while the 3 ceramide ratios predict the composite endpoint of death and ACS. These associations were independent of statin use and LDL cholesterol level.

Ceramides are a family of waxy lipid molecules and are composed of sphingosine and a fatty acid. Experimental studies have shown that ceramides and related sphingolipids are associated with the development of atherosclerosis [5,20]. Ceramides, especially lactosylceramide and glucosylceramide, accumulate in the atherosclerotic plaque [5,21], and have been shown to suppress production of apolipoprotein E leading to an accumulation of cholesterol in macrophage foam cells [5,22]. Inhibition of the glycosphingolipid pathway was shown to decrease atherosclerosis in mice [5,23]. Several enzymes in the ceramide synthetic pathway have been tested as potential drug targets in animal models [5,24,25].

This study investigated eight molecular lipids and three ceramide ratios that were associated with fatal cardiovascular outcome at the p < 0.001 level in the LURIC lipidomic study, which compared 158 CAD patients who died within 3 years of follow-up with 187 matched control patients with CAD who did not die during follow-up [5]. The following findings in this study support the hypothesis that specific ceramide molecules play an important role in development of atherosclerosis and plaque vulnerability: 1. we have found that the plasma concentration of the majority of the evaluated lipids are higher in ACS patients than in patients with stable CAD; and 2. we have found that the plasma concentration of the majority of the evaluated lipids were associated with percentage necrotic core tissue and lipid core burden as assessed by IVUS-VH and NIRS imaging, and 3. we have confirmed that Cer(d18:1/16:0) and the three evaluated ceramide ratios predict cardiovascular outcome.

Although serum LDL cholesterol is an established cardiovascular risk factor, previous IVUS studies have failed to demonstrate a strong association between LDL and coronary plaque burden [26]. On the other hand, a recent study showed that high-intensity LDL lowering statin therapy is associated with regression of coronary

Table 3
Significant associations with coronary plaque characteristics.

	Plaque burden		Fibrous tissue		Fibro-fatty tissue		Dense calcium		Necrotic core		LCBI[a]	
	Δ (%)	P	Δ (%)	P	Δ (%)	P	Δ (%)	P	Δ (%)	P	Δ (%)	P
CE 14:0	−14.4	0.015	0.8	0.86	−6.1	0.31	4.7	0.39	7.6	0.087	7.6	0.17
CE 18:3	−7.1	0.19	1.2	0.61	−8.0	0.17	−0.1	0.81	10.7	0.040	9.9	0.13
CE 20:5	4.3	0.61	−0.3	0.93	−7.9	0.48	11.7	0.15	19.7	0.022	13.3	0.26
Cer(d18:1/16:0)	−1.6	0.52	−1.8	0.48	−11.0	0.001	3.4	0.42	13.5	0.001	11.5	0.024
Cer(d18:1/24:0)	−4.6	0.15	−0.6	0.99	−11.6	0.010	−1.4	0.59	14.6	0.003	24.6	0.001
LacCer(d18:1/18:0)	4.6	0.45	−3.8	0.56	−9.9	0.029	7.3	0.30	8.4	0.14	12.7	0.049
Cer(d18:1/16:0)/Cer(d18:1/24:0)	5.8	0.21	−3.7	0.39	−1.4	0.78	6.8	0.084	1.4	0.90	−8.9	0.039
Cer(d18:1/20:0)/Cer(d18:1/24:0)	10.8	0.036	−8.1	0.040	−4.8	0.29	10.9	0.009	3.1	0.60	−6.4	0.27

Presented data are relative differences in mean lipid concentration between the highest quartile and the lowest quartile of 1. plaque burden; 2. fibrous tissue percentage; 3. fibrofatty tissue percentage; 4. dense calcium percentage; 5. necrotic core percentage; and 6. LCBI.
[a] Lipid core burden index (LCBI) was measured in 191 patients.

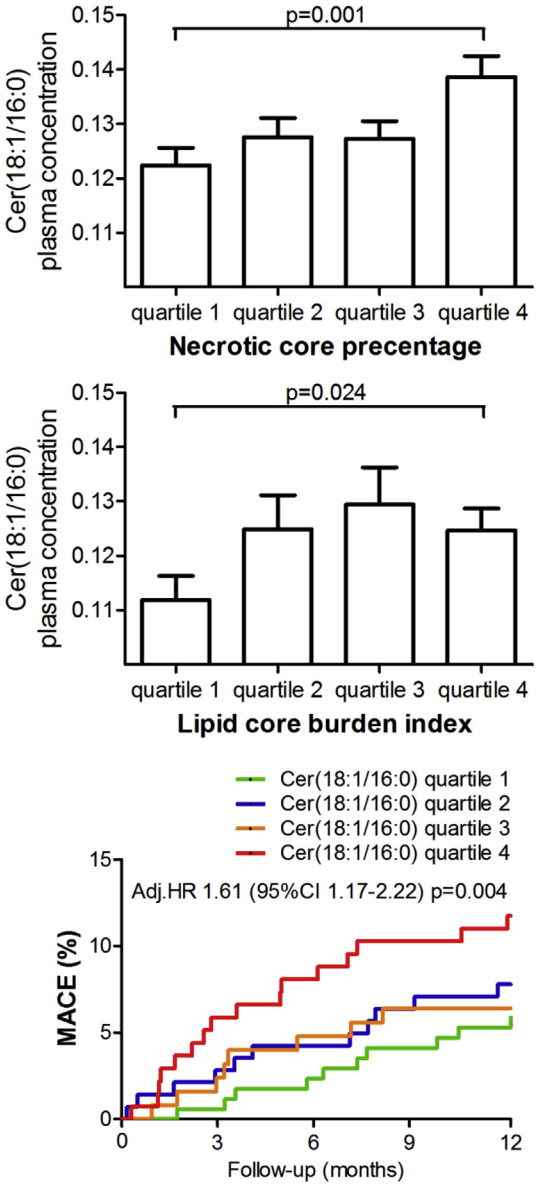


Fig. 2. Association of plasma ceramide concentration with coronary plaque morphology and cardiovascular outcome.

atherosclerosis in non-infarct-related arteries without changes in IVUS defined necrotic core or plaque phenotype among STEMI patients [27]. To the best of our knowledge, we are the first to demonstrate the association between molecular lipid species and coronary plaque characteristics using in-vivo imaging. Future studies are required to investigate whether therapy that reduce the concentration of these high-risk molecular lipids also lead to regression of plaque burden and regression of high-risk plaque phenotype.

Some limitations of this study need to be acknowledged. Firstly, a single non-culprit coronary vessel was imaged in this study. This approach was eventually chosen to test the hypothesis that the phenotype of a non-culprit artery segment may indicate the patient's systemic atherosclerotic disease burden [12]. This hypothesis is supported by our previous findings that IVUS-VH and NIRS imaging in only one non-culprit vessel are associated with prognosis [11]. However, necrotic core-rich and lipid core-rich plaques elsewhere in the coronary tree (including the culprit lesion) were not assessed in our study. This may have led to an underestimation of the association between plasma lipids and vulnerable plaque morphology in the coronary tree. Secondly, half of the study patients had an ACS, while the other half had stable CAD. This may have influenced the study results, because the molecular lipid profile may have changed during ACS. There are no serial data available showing the course of lipid levels during ACS, and therefore the change in lipid concentration during such an acute thrombotic event is unknown. Furthermore, because of lack of statistical power, we could not assess whether the association between molecular lipid levels and plaque characteristics were similar in ACS and stable CAD. Finally, this single center study is primarily designed to evaluate the associations between high risk lipids and IVUS-VH. The number of clinical endpoints was relatively small. Thus, we may have had insufficient power to detect significant associations between some of the molecular lipids and occurrence of MACE during follow-up.

In conclusion, plasma concentrations of several cholesteryl ester, ceramide and lactosylceramide species were associated with the fraction of necrotic core tissue on IVUS-VH imaging and with the lipid core burden on NIRS imaging of coronary atherosclerosis. Some of these molecular lipid species, and particularly ceramide(d18:1/16:0), were strongly associated with 1-year clinical outcome after coronary angiography, independently from statin use and LDL cholesterol levels. The three investigated ceramide ratios predicted the composite endpoint of death and ACS. Our results confirm the findings of previous lipidomic analysis and further supports the associations of ceramide plasma concentrations and ratios with fatal outcome by demonstrating associations with coronary plaque vulnerability. Circulating molecular lipids may potentially be used to improve risk stratification in patients with CAD and might also be interesting therapeutic targets for the treatment of atherosclerotic disease.

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Table 4
Significant associations with cardiovascular outcome.

	MACE		Death or ACS	
	HR (95%CI)[a]	P	HR (95%CI)[a]	P
Cer(d18:1/16:0)	1.61 (1.17–2.22)	0.004	1.88 (1.24–2.84)	0.003
Cer(d18:1/16:0)/Cer(d18:1/24:0)	1.18 (0.88–1.57)	0.28	1.56 (1.13–2.14)	0.007
Cer(d18:1/20:0)/Cer(d18:1/24:0)	1.10 (0.81–1.50)	0.54	1.54 (1.06–2.24)	0.023
Cer(d18:1/24:1)/Cer(d18:1/24:0)	1.49 (1.08–2.05)	0.014	1.84 (1.24–2.72)	0.002

Data are presented as HR per standard deviation increase in log-transformed lipid concentration. Definite culprit lesion-related events were not counted as endpoint.
ACS, acute coronary syndrome; HR, hazard ratio; MACE, major adverse cardiac event.
[a] Adjusted for age, gender, diabetes, statin use and clinical presentation (ACS or stable coronary artery disease).

Disclosures

Matti Suoniemi, Terhi Vihervaara, Marko Sysi-Aho, Kim Ekroos and Reijo Laaksonen are employed by Zora Biosciences, Espoo, Finland. Other authors declare no conflict of interest.

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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.2015.10.022.

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