Atherosclerosis xxx (xxxx) 1–11

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

A specific plasma lipid signature associated with high triglycerides and low HDL cholesterol identifies residual CAD risk in patients with chronic coronary syndrome

Nicoletta Di Giorgi [a, #], Elena Michelucci [a, #], Jeff M. Smit [b], Arthur J.H.A. Scholte [b], Mohammed El Mahdiui [b], Juhani Knuuti [c], Ronny R. Buechel [d], Anna Teresinska [e], Maria N. Pizzi [f], Albert Roque [g], Rosa Poddighe [h], Oberdan Parodi [i], Gualtiero Pelosi [a], Chiara Caselli [a, **], Danilo Neglia [i, 1], Silvia Rocchiccioli [a, 1, *]

[a] *Istituto di Fisiologia Clinica-CNR, via Giuseppe Moruzzi 1, 56124, Pisa, Italy*
[b] *Department of Cardiology, Leiden University Medical Center, Albinusdreef 2, 2333, Leiden, ZA, the Netherlands*
[c] *Turku PET Centre, Turku University Hospital and University of Turku, 20520, Turku, Finland*
[d] *Department of Nuclear Medicine, Cardiac Imaging, University Hospital and University of Zurich, Switzerland*
[e] *National Institute of Cardiology, Warsaw, Poland*
[f] *Department of Cardiology, Hospital Universitari Vall d'Hebron, Barcelona, Spain*
[g] *Department of Radiology, Hospital Universitari Vall d'Hebron, Barcelona, Spain*
[h] *ASL12 U.O.C, Cardiologia, Viareggio, Italy*
[i] *Fondazione Toscana Gabriele Monasterio, Viale Giuseppe Moruzzi 1, Pisa, 56124, Italy*

ARTICLE INFO

Keywords:
Triglycerides
HDL-C
Coronary artery disease
Lipidomics
Coronary CTA

ABSTRACT

Background and aims: Elevated triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) define a specific lipid profile associated with residual coronary artery disease (CAD) risk independently of total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels. Aim of the present study was to assess whether TG/HDL-C ratio, coronary atherosclerosis and their change over time are characterized by a specific lipidomic profiling in stable patients with chronic coronary syndrome (CCS).

Methods: TG/HDL-C ratio was calculated in 193 patients (57.8 ± 7.6 years, 115 males) with CCS characterized by clinical, bio-humoral profiles and cardiac imaging. Patient-specific plasma targeted lipidomics was defined through a high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) strategy. Patients underwent coronary computed tomography angiography (CTA) and an individual CTA risk score, combining extent, severity, composition, and location of plaques, was calculated. All patients entered a follow-up (6.39 ± 1.17 years), including clinical, lipidomics and coronary CTA assessments.

Results: Patients were divided in groups according to baseline TG/HDL-C quartiles: IQ (<1.391), IIQ (1.392–2.000), IIIQ (2.001–3.286), and IVQ (≥3.287). A specific pattern of altered lipids, characterized by reduced plasma levels of cholesterol esters, phosphatidylcholines and sphingomyelins, was associated with higher TG/HDL-C both at baseline and follow-up (IVQ *vs* IQ). The CTA risk score increased over time and this lipid signature was also associated with higher CTA score at follow-up.

BMI, Body mass index; CAD, Coronary artery disease; CCS, Chronic coronary syndrome; CE, Cholesterol ester; Cer, Ceramide; CTA, Computed tomography angiography; CTA score, Comprehensive coronary atherosclerotic risk score; DMS, N,N-dimethylsphingosine (d18:1); FPG, Fasting plasma glucose; HDL-C, High-density lipoprotein cholesterol; HPLC-MS/MS, High performance liquid chromatography-tandem mass spectrometry; LDL-C, Low-density lipoprotein cholesterol; non-HDL-C, Non-high-density lipoprotein cholesterol; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PS, Phosphatidylserine; RCT, Reverse cholesterol transport; SM, Sphingomyelin; SRM, Selected reaction monitoring; Total-C, Total-cholesterol; TG, Triglycerides

* Corresponding author.
** Corresponding author.
E-mail addresses: chiara.caselli@ifc.cnr.it (C. Caselli), silvia.rocchiccioli@ifc.cnr.it (S. Rocchiccioli).
[1] These two authors are co-last authors.
[#] These two authors contribute equally to this work.

https://doi.org/10.1016/j.atherosclerosis.2021.11.013
Received 29 July 2021; Received in revised form 20 October 2021; Accepted 9 November 2021

Conclusions: In stable CCS, a specific lipidomic signature identifies those patients with higher TG/HDL- C ratio and higher CTA score over time, suggesting possible molecular pathways of residual CAD risk not tackled by current optimal medical treatments.

1. Introduction

Coronary artery disease (CAD), is a chronic and progressive disease, characterized by atherosclerotic plaque accumulation in the epicardial arteries. Despite the remarkable progress in pharmacological and interventional treatments, CAD remains a major cause of death [1] also due to "residual risk" associated with specific cardiometabolic profiles, which is not tackled by current treatments. High triglyceride and low high-density lipoprotein cholesterol levels, as simplified by the TG/HDL-C ratio, identify one of this emerging cardiometabolic risk profiles characterized by insulin-resistance, obesity and metabolic syndrome known to be associated with angiographically documented CAD [2–11] and CAD related outcomes [12].

Besides classical lipid profile, lipidomics is a systems-based study of all lipids. Specifically, lipidomics is able to quantify diverse molecular lipid species across multiple lipid classes such as sphingolipids, phospholipids, sterol esters and acylglycerols, many of which play an integral role in modulation of biological functions such as formation of cellular membranes, energy storage and cell signaling [13]. Plasma lipidomic profile assessed by mass spectrometry is gaining increasing importance as a source of putative biomarkers of plaque composition and vulnerability, which could acquire clinical relevance in the future.

Growing evidence suggests that specific lipid species could be predictors of CAD related risk and of adverse events [14–19]. Some reports also support a close association between dysregulation of lipid species and atherosclerotic lipid burden in patients on standard of care cholesterol lowering treatment [15].

The aim of the present study was to use targeted lipidomics analysis of CAD-related circulatory lipid species [20] to assess whether a specific lipidomic signature characterizes the cardiometabolic risk profile identified by higher TG/HDL-C ratio and whether both are related to coronary atherosclerotic risk evolution over time, as evaluated by repeated coronary computed tomography angiography (CTA) in a population of stable patients with chronic coronary syndrome (CCS).

2. Materials and methods

2.1. Study design, patient population and ethical approval

The study population was identified within the SMARTool (Simulation Modeling of coronary Artery disease: a tool for clinical decision support) study [20–25]. In brief, stable patients with CCS undergoing baseline coronary CTA as part of the EVINCI (Evaluation of Integrated Cardiac Imaging for the Detection and Characterization of Ischemic Heart Disease; FP7-222915) or ARTreat (FP7-224297) studies, were prospectively enrolled in the SMARTool study and submitted to clinical, molecular and coronary CTA re-evaluation at follow-up (interscan period 6.39 ± 1.17 y). For all patients, blood samples at fasting state were collected before each coronary CTA exam and were stored in the Clinical Physiology Institute Biobank.

Among 263 patients undergoing follow-up coronary CTA, 193 had also baseline and follow-up blood samples, which allowed lipidomics analyses and were included in the current sub-study.

The SMARTool study was conducted according to the Declaration of Helsinki and its later amendments. It was coordinated by the Clinical Physiology Institute in Pisa and included 7 European countries (Clinicaltrial.gov Identifiers NCT0444869). The protocol was approved by the Coordinating Center Ethical Committee and all local Ethical Committees. All patients included in the study signed a written informed consent.

2.2. Clinical and bio-humoral characteristics

Information on cardiovascular risk factors, including age, gender, symptoms, family history of CAD, smoking status, diabetes, dyslipidemia, hypertension, obesity, medication use and bio-humoral profiles, was collected at baseline and follow-up in all patients. Diabetes was defined as fasting plasma glucose (FPG) > 126 mg/dL or treatment with glucose lowering medications; dyslipidemia as low-density lipoprotein cholesterol (LDL-C) > 120 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL for men or < 50 mg/dL for women, triglycerides (TG) > 150 mg/dL or current treatment with lipid-lowering medication; hypertension as systolic and diastolic pressure > 140/90 mmHg or treatment; obesity as body mass index (BMI) > 30 kg/m^2 and/or waist circumference > 102 cm for men or > 88 cm for women.

Biochemical markers associated with glucose and lipid metabolism were determined at baseline and follow-up in all 193 patients. FPG, total-cholesterol (total-C), TG, and HDL-C levels (mg/dL) were evaluated using standard methods and ranges of normality previously reported [26–28]. LDL-C was calculated using the Friedewald formula [29].

Non-high-density lipoprotein cholesterol (non-HDL-C) was calculated as total-C minus HDL-C. The TG/HDL-C ratio was calculated as TG level divided by HDL-C level. Clinical, imaging and lipidomic variables were analysed according to TG/HDL-C cut-off values defined according to baseline TG/HDL-C quartiles.

2.3. Coronary computed tomography angiography

Coronary CTA scans were obtained using > 64-slice scanners and were in line with the technical requirements defined in the SMARTool Study to ensure optimal image quality. All CTA images were analysed blinded to clinical data by a separate Core Laboratory (Leiden University Medical Center) and coronary arteries were assessed according to the modified 17-segment American Heart Association classification [30].

Obstructive CAD was defined in the presence of > 50% stenosis in at least one major coronary vessel. Non obstructive CAD, minimal CAD and no CAD were defined in the presence of 30–50% stenosis, < 30% stenosis and no stenosis, respectively [31].

Each segment of the AHA 17-coronary segment model was assessed for interpretability, and interpre Supplementary Table segments were evaluated for the degree of stenosis of the coronary artery and plaque composition. If a plaque was present, plaque composition was visually determined (calcified, non-calcified, and mixed). Only one type of plaque composition could be assigned to a single segment. A comprehensive coronary atherosclerotic risk score (CTA score), previously validated as a predictor of adverse events (all cause death or non-fatal MI) in patients without known CAD, was derived from CTA scans by integration of all data on the location, severity and composition (calcified, non-calcified, and mixed plaque) of coronary plaques [32]. All patients with interpretable CTA scan both at baseline and follow-up were classified into 3 groups (low, intermediate and high) using CTA score severity thresholds (< 5, 5–20 and > 20), as previously reported [33]. Additionally, patients were stratified matching the CTA score groups of belonging at baseline (low, intermediate and high) with those at follow-up (low, intermediate and high), thus identifying patients without modifications of CTA score group from baseline to follow-up (low-low, intermediate-intermediate, high-high); patients, who developed CTA progression, with the shift from CTA score groups at baseline to the more severe group at follow-up (from low to intermediate, from intermediate to high); and patients (n = 5) who showed a regression of the disease

(3 from hoow CTA score group), who were excluded from the analysis, since their small number would not have allowed a rigorous statistical evaluation. Finally, considering also the severity of disease, 5 categories of CTA score group combinations were ordered from the low to the high CAD risk: low-low, low-intermediate, intermediate-intermediate, intermediate-high, high-high.

2.4. Plasma sample preparation and HPLC-MS/MS targeted lipidomics 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 Folch procedure [34]: 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) (DMS) in MeOH/CHCl3 1/2 v/v. 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, 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 (SRM)-based HPLC-MS/MS method, as previously reported in Michelucci, E. et al. and related Supplementary Information [20].

2.5. Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD), categorical variables as numbers and relative percentages. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess how close the data were to a normal distribution. For descriptive purpose, when baseline and follow-up were considered, means were compared using Wilcoxon rank sum test. Alternatively, means were compared across multiple groups using Kruskal-Wallis test, followed by two-tailed Mann Whitney U-test with Bonferroni correction only for the variables resulted significantly different (Kruskal-Wallis p-value < 0.05). Specifically, Mann Whitney U- test allowed to compare extreme groups. Percentages were compared between groups using Chi-square test or Fisher's exact test for small-sized categories. Clinical data were analyzed using SPSS Statistics software (IBM, version 26).

Quantitative data of 51 lipid species from targeted lipidomics analyses were compared among groups defined by TG/HDL-C or CTA score cut-off values. For each lipid species differences in plasma levels were evaluated as fold change (FC), defined by ratio of mean plasma concentration in different groups to that of the reference group (lower TG/HDL-C or CTA score). As the data were not normally distributed (p-values <0.05), two-tailed Mann Whitney U-test was performed to consider the significant differences in lipid levels. p-values were corrected using the Benjamini-Hochberg procedure to minimize any type I error and thus the occurrence of false positives. Lipids differences were considered statistically significant with a corrected p-value lower than 0.05 for all analysis. Lipidomics experimental data were analyzed using R software (version 3.6.3).

To examine conditional correlations ($r \geq 0.25$) between lipid species and traditional CAD lipid biomarkers, partial correlation coefficients were calculated for each of 51 lipid species and conventional lipids at baseline and follow-up. Respective visualizations were generated in Cytoscape software (version 3.8.0).

3. Results

3.1. Study population at baseline and follow-up

Clinical characteristics, treatments, bio-humoral profiles, coronary CTA results and CTA risk score at baseline and after 6 year follow-up are summarized in Table 1. Prevalence of dyslipidemia, hypertension,

Table 1
Features of patient population at baseline and follow-up.

	Baseline	Follow-up	p-value
N	193	193	–
Age (yrs)	57.8 ± 7.6	64.1 ± 7.7	*** <0.001
Males	115 (59.6%)	115 (59.6%)	–
Risk factors			
Family history	91 (47.2%)	91 (47.2%)	–
Smoking[a]	32 (16.6%)	21 (10.9%)	0.139
Diabetes mellitus[a]	41 (21.2%)	54 (28.0%)	0.156
Dyslipidemia[a]	119 (61.7%)	146 (75.6%)	** 0.004
Hypertension[a]	119 (61.7%)	142 (73.6%)	* 0.017
Obesity[a]	37 (19.2%)	49 (25.4%)	0.178
Medications			
Statin	99 (51.3%)	128 (66.3%)	** 0.004
Antidiabetics	33 (17.1%)	50 (25.9%)	* 0.048
Antihypertensives	97 (50.3%)	111 (57.5%)	0.184
Anti-ischemics	89 (46.1%)	92 (47.7%)	0.838
Antiplatelets	125 (64.8%)	110 (57.0%)	0.144
Calcium antagonists or nitrates	40 (20.7%)	52 (26.9%)	0.189
Anticoagulants	1 (0.5%)	10 (5.2%)	* 0.011
Glucose and lipid profile			
FPG (mg/dL)	107.8 ± 25.7	106.0 ± 24.1	0.495
Total cholesterol (mg/dL)	184.1 ± 49.0	179.5 ± 45.3	0.170
LDL-C(mg/dL)	109.7 ± 40.7	95.3 ± 38.6	*** <0.001
HDL-C(mg/dL)	52.1 ± 16.4	57.0 ± 17.3	*** <0.001
non-HDL-C (mg/dL)	132.1 ± 43.6	122.5 ± 39.0	** 0.006
Triglycerides (mg/dL)	114.9 ± 56.5	138.2 ± 73.0	*** <0.001
TG/HDL-C ratio	2.5 ± 1.8	2.8 ± 2.3	0.085
CAD at CTA			
No[a]	43 (22.3%)	30 (15.5%)	0.128
Minimal[a]	65 (33.7%)	58 (30.1%)	0.528
Non-obstructive[a]	32 (16.6%)	56 (29.0%)	* 0.011
Obstructive[a]	48 (24.9%)	45 (23.3%)	0.756
CTA score	10.8 ± 9.6	13.0 ± 9.4	*** <0.001
CAD-related events			
AMI	1 (0.5%)	0 (0.0%)	1.000
PCI[b]	2 (1.0%)	28 (14.5%)	*** <0.001
CABG[b]	1 (0.5%)	9 (4.7%)	* 0.020

*$p < 0.05$, **$p < 0.01$, *** p- < 0.001.
Continuous variables are presented as mean ± standard deviation (SD), categorical variables as numbers and relative percentages. For descriptive purpose, means were compared using Wilcoxon rank sum test, while percentages were compared using Chi-square test or Fisher's exact test for small-sized categories. AMI, acute myocardial infarction; CABG, coronary artery bypass graft; CTA score, comprehensive atherosclerotic risk score based on non-invasive CCTA coronary imaging; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCI, percutaneous coronary intervention; TG, triglycerides; TG/HDL-C ratio, triglyceride to high-density lipoprotein cholesterol ratio.

[a] Clinical variables are defined as follow: Smoking, current smoking; diabete mellitus, Fasting plasma glucose >126 mg/dL and/or GHbA1c > 6.5% or under treatment; dyslipidemia, LDL-C > 120 mg/dL, HDL-C < 40 for man or <50 for woman, triglycerides >150 mg/dL or under specific treatments; hypertension, systolic and diastolic pressure >140/90 mmHg or under treatment; Obesity, BMI Kg/m^2 > 30 and/or waist circ >102 cm for man or > 88 cm for woman. Obstructive, non obstructive, minimal and no CAD were defined in the presence of >50% stenosis, 30–50% stenosis, < 30% stenosis and no stenosis respectively.

[b] Patients with coronary revascularization were included in the study only if PCI or CABG occurred more than 6 months from their enrolment.

use of statins, antidiabetics, anticoagulants and frequency of coronary revascularization (either by PCI or CABG) increased from baseline to follow-up. The classical lipid profile showed a significant increase of TGs (p-values < 0.001) with a borderline increase in TG/HDL-C ratio ($p = 0.066$) from baseline to follow-up. HDL-C increased while non-HDL-C and LDL-C significantly decreased, without differences in Total-C. The prevalence of obstructive CAD remained stable at around 24% as well as that of minimal CAD, while the prevalence of no CAD decreased from 22.3% to 15.5% and that of non-obstructive CAD showed a significant increase from 16.6% to 29.0% (p-values 0.011). As shown in Fig. 1, CTA score at follow-up was positively correlated with CTA at baseline (r = 0.90, p-value < 0.001) and significantly increased from 10.8 ± 9.6 at baseline to 13.0 ± 9.4 at follow-up (p-value < 0.001). The progression of disease according to change of CTA score groups from baseline to follow-up is reported in Fig. 1. Out of the 62 patients in the Low CTA group at baseline, 21 (32.3%) suffered from a worsening of disease, changing respectively from Low to Intermediate CTA score group. Again, out of the 90 patients in the Intermediate CTA group at baseline, 15 (16.7%) shifted from Intermediate to High CTA score group. Almost all patients (31/34, 91.2%) with High CTA score at baseline remained patients at high risk. Overall, while only 2.6% of the population showed an improvement in CAD status, 19% showed a progression of CAD.

3.2. TG/HDL-C, bio-humoral and CTA characteristics

In the overall study population, the mean baseline value of TG/HDL-C ratio was 2.5 ± 1.8 (mean ± SD) and thresholds for TG/HDL-C groups were defined as follows: TG/HDL-C < 1.391 (I Quartile); TG/HDL-C 1.392–2.000 (II Quartile); TG/HDL-C 2.001–3.286 (III Quartile); TG/HDL-C ≥ 3.287 (IV Quartile).

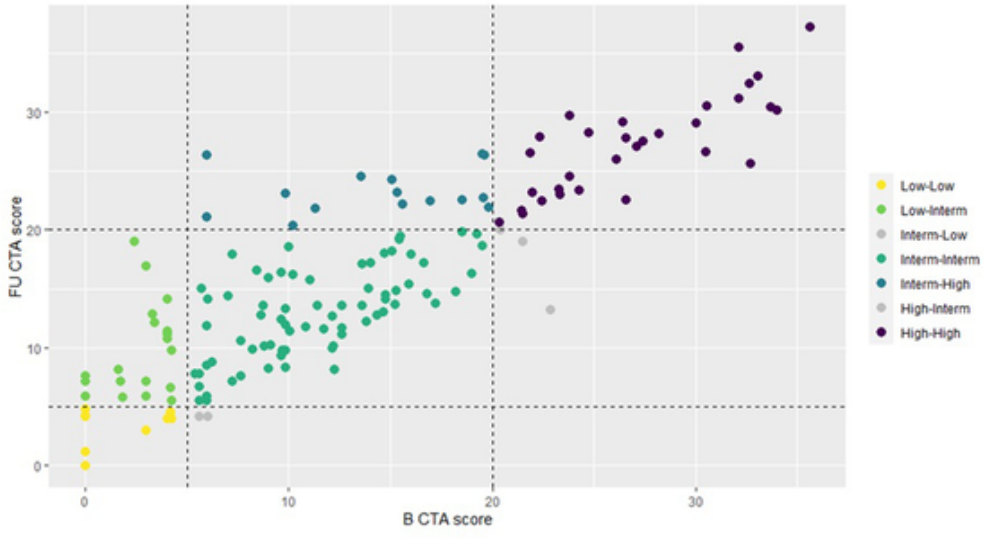
According to TG/HDL-C ratio cut-offs, the baseline and follow-up clinical characteristics and biochemical parameters of patients are listed in Table 2. Male gender and diabetes were more frequent in the IVQ as compared with the IQ group of TG/HDL-C ratio. Obesity displayed a significant trend to increase across TG/HDL-C groups at follow-up.

Both at baseline and follow-up, the different TG/HDL-C groups were characterized by progressively higher TG and progressively lower HDL-C (p-values < 0.001). Total-C and LDL-C did not show a progressive trend at baseline while were progressively lower among TG/HDL-C groups at follow-up. Non-HDL-C showed a significant difference across TG/HDL-C groups at baseline but did not show consistent trend at follow-up.

At baseline, CAD was present in 36.7% of patients in the IQ TG/HDL-C group with a significant decrease across TG/HDL-C groups. The prevalence of obstructive CAD was not different among TG/HDL-C groups while the CTA score increased across TG/HDL-C groups both at baseline and follow-up (p-values = 0.012 and 0.017 respectively, refer to Supplementary Fig. S1 for details).

3.3. TG/HDL-C and targeted lipidomics

Data analysis of 51 lipid species encompassing seven lipid classes and subclasses (cholesterol ester [CE], ceramide [Cer], phosphatidylcholine [PC], phosphatidylethanolamine [PE], phosphatidylserine [PS], sphingomyelin [SM], triacylglycerol [TG]) was performed with the aim of identifying lipid molecules associated with cardio-metabolic



Follow-up CTA Score groups

Baseline CTA Score groups	Low	Interm	High	
Low	44	21	0	65
Interm	2	73	15	90
High	0	3	31	34
	46	97	46	189

Fig. 1. Plot showing the positive correlation (r = 0.90, $p < 0.001$) between follow-up and baseline CTA score in our population.
For both axes, dashed lines indicate CTA score values of 5 and 20, according to thresholds (< 5, 5–20 and > 20) used to classify patients into Low, Intermediate and High risk. Dots represent patients, which are in colour according to the stratification by the evolution of relative CTA score groups from baseline to follow-up in 5 categories [Low-Low (yellow), Low-Intermediate (light green), Intermediate-Intermediate (dark green), Intermediate-High (light blue), High-High (dark blue)]. Five patients who showed a regression of the disease (3 from High to Intermediate and 2 from Intermediate to Low CTA score group) are in grey. The relative composition of categories is shown in the table below. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Patients features according to baseline TG/HDL-C ratio at both baseline and follow-up.

		I Q TG/HDL-C <1.391	II Q TG/HDL-C 1.392–2.000	III Q TG/HDL-C 2.001–3.286	IV Q TG/HDL-C ≥3.287	*p*-value	IV *vs* I Q *p* value
N	B	49	48	48	48	–	–
	FU	34	48	66	45	–	–
Age (yrs)	B	57.3 ± 7.7	58.4 ± 7.3	59.9 ± 7.0	55.5 ± 8.0	* 0.040	n.s.
	FU	63.8 ± 5.4	63.8 ± 8.6	65.2 ± 8.0	63.1 ± 7.7	0.557	–
Males	B	23 (46.9%)	23 (47.9%)	30 (62.5%)	39 (81.2%)	* 0.001	*** <0.001
	FU	15 (44.1%)	24 (50.0%)	44 (66.7%)	32 (71.1%)	* 0.028	* 0.016
Family history	B	24 (49.0%)	24 (50.0%)	23 (47.9%)	20 (41.7%)	0.846	–
	FU	16 (47.1%)	26 (54.2%)	33 (50.0%)	16 (35.6%)	0.309	–
Smoking	B	6 (12.2%)	7 (14.6%)	5 (10.4%)	14 (29.2%)	0.055	–
	FU	1 (2.9%)	4 (8.3%)	9 (13.6%)	7 (15.6%)	0.255	–
Diabetes mellitus	B	5 (10.2%)	15 (31.2%)	5 (10.4%)	16 (33.3%)	** 0.003	** 0.006
	FU	4 (11.8%)	11 (22.9%)	24 (36.4%)	15 (33.3%)	* 0.046	* 0.026
Dyslipidemia	B	27 (55.1%)	34 (70.8%)	30 (62.5%)	28 (58.3%)	0.417	–
	FU	26 (76.5%)	34 (70.8%)	50 (75.8%)	36 (80.0%)	0.782	–
Hypertension	B	24 (49.0%)	30 (62.5%)	29 (60.4%)	36 (75.0%)	0.072	–
	FU	24 (70.6%)	35 (72.9%)	48 (72.7%)	35 (77.8%)	0.896	–
Obesity	B	4 (8.2%)	8 (16.7%)	9 (18.8%)	16 (33.3%)	* 0.017	* 0.002
	FU	6 (17.6%)	7 (14.6%)	20 (30.3%)	16 (35.6%)	0.062	–
Statin users	B	28 (57.1%)	23 (47.9%)	23 (47.9%)	25 (52.1%)	0.772	–
	FU	20 (58.8%)	30 (62.5%)	46 (69.7%)	32 (71.1%)	0.579	–
FPG (mg/dL)	B	100.4 ± 18.3	113.7 ± 26.0	104.0 ± 23.4	113.0 ± 31.3	** 0.005	n.s.
	FU	100.2 ± 11.0	101.9 ± 12.3	105.3 ± 26.8	115.7 ± 32.8	0.226	–
Total cholesterol (mg/dL)	B	176.0 ± 48.0	192.7 ± 48.8	191.5 ± 49.3	176.5 ± 48.9	0.138	–
	FU	198.3 ± 46.3	185.9 ± 50.3	173.8 ± 42.8	167.0 ± 37.4	* 0.016	* 0.020
LDL-C (mg/dL)	B	98.9 ± 40.0	117.7 ± 37.8	120.6 ± 41.0	101.9 ± 40.8	** 0.008	n.s.
	FU	103.6 ± 37.9	103.3 ± 42.2	95.5 ± 39.8	79.3 ± 28.0	* 0.017	* 0.031
HDL-C (mg/dL)	B	64.7 ± 18.7	56.3 ± 11.8	47.2 ± 10.9	39.7 ± 11.0	*** <0.001	*** <0.001
	FU	79.5 ± 18.4	61.3 ± 13.1	52.2 ± 9.1	42.4 ± 9.1	*** <0.001	*** <0.001
non-HDL-C (mg/dL)	B	111.3 ± 40.0	136.4 ± 39.8	144.2 ± 43.2	136.8 ± 45.2	** 0.001	* 0.020
	FU	118.8 ± 38.5	124.6 ± 43.9	121.6 ± 40.0	124.6 ± 33.0	0.831	–
Triglycerides (mg/dL)	B	61.8 ± 16.7	94.3 ± 22.2	120.9 ± 29.1	183.5 ± 56.7	*** <0.001	*** <0.001
	FU	75.5 ± 20.0	102.8 ± 20.5	131.8 ± 26.0	232.7 ± 88.9	*** <0.001	*** <0.001
CTA score	B	8.4 ± 9.7	11.0 ± 9.8	9.9 ± 9.2	13.8 ± 9.0	* 0.012	** 0.007
	FU	9.2 ± 8.1	12.1 ± 10.0	13.9 ± 8.9	15.6 ± 9.6	* 0.017	* 0.017
CAD							
No	B	18 (36.7%)	12 (25.0%)	9 (18.8%)	4 (8.3%)	* 0.008	* 0.001
	FU	10 (29.4%)	7 (14.6%)	6 (9.1%)	7 (15.6%)	0.069	–
Minimal	B	17 (34.7%)	10 (20.8%)	17 (35.4%)	21 (43.8%)	0.121	–
	FU	11 (32.4%)	13 (27.1%)	24 (36.4%)	10 (22.2%)	0.415	–
Non-obstructive	B	5 (10.2%)	13 (27.1%)	6 (12.5%)	8 (16.7%)	0.119	–
	FU	6 (17.6%)	15 (31.2%)	18 (27.3%)	17 (37.8%)	0.259	–
Obstructive	B	9 (18.4%)	13 (27.1%)	12 (25.0%)	14 (29.2%)	0.635	–
	FU	7 (20.6%)	10 (20.8%)	17 (25.8%)	11 (24.4%)	0.906	–

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
Continuous variables are presented as mean ± standard deviation (SD), categorical variables as numbers and relative percentages. Means were compared across multiple groups using Kruskal-Wallis test, followed by a or two-tailed Mann Whitney U-test with Bonferroni correction only for the variables resulted significantly different (Kruskal-Wallis p-value < 0.05). Specifically, Mann Whitney U-test allowed to compare the extreme group of subjects (IV Q) with respect to the control group (I Q). Percentages were compared between groups using Chi-square test or Fisher's exact test for small-sized categories.

risk represented by TG/HDL-C ratio. The baseline and follow-up lipidomic profiles are compared among groups defined by TG/HDL-C cut-off values (Fig. 2). At baseline 20 lipid species were significantly altered in the IV Quartile *vs* the I Quartile of TG/HDL-C ratio: plasma levels of 8 SMs [SM(34:1), SM(36:2), SM(38:2), SM(38:1), SM(40:2), SM (41:2), SM(42:4), SM(42:3)], 3 CEs [CE(16:0), CE(18:0), CE(18:1)) and 3 PCs [PC(38:2), PC(36:1e)/PC(36:0p), PC(36:2e)/PC(36:1p)] were decreased, with FC values between 0.6 and 0.9, while plasma levels of all TG species analysed [TG(50:1), TG(50:2), TG(52:2), TG(52:3), TG (54:2), TG(54:3)] were increased, with FC values between 1.7 and 3.6. While increased plasma levels of TGs were already evident, even if with lower FC, comparing II and III *vs* I Quartile, decreased lipid species were only evident in the IV *vs* the I Quartile (Fig. 2A, Supplementary Table 1). At follow-up, 21 lipid species were significantly altered in the IV Quartile *vs* the I Quartile: plasma levels of 8 SMs [SM(36:2), SM (38:2), SM(40:2), SM(41:2), SM(41:1), SM(42:4), SM(42:3), SM(42:1)], 3 CEs [CE(16:0), CE(18:0), CE(18:1)], PE(38:1) and 2 PCs [PC(38:2), PC(36:2e)/PC(36:1p)] were decreased, with FC values between 0.6 and 0.9, while plasma levels of PS(36:1) and all TG species analysed were increased, with FC values between 1.7 and 5.3. As compared with baseline, increased plasma levels of TGs and decreased plasma levels of SM, CE and PC lipid species were more evident, reaching significance also in lower quartiles and showing higher between-group differences (Fig. 2B and Supplementary Table 1).

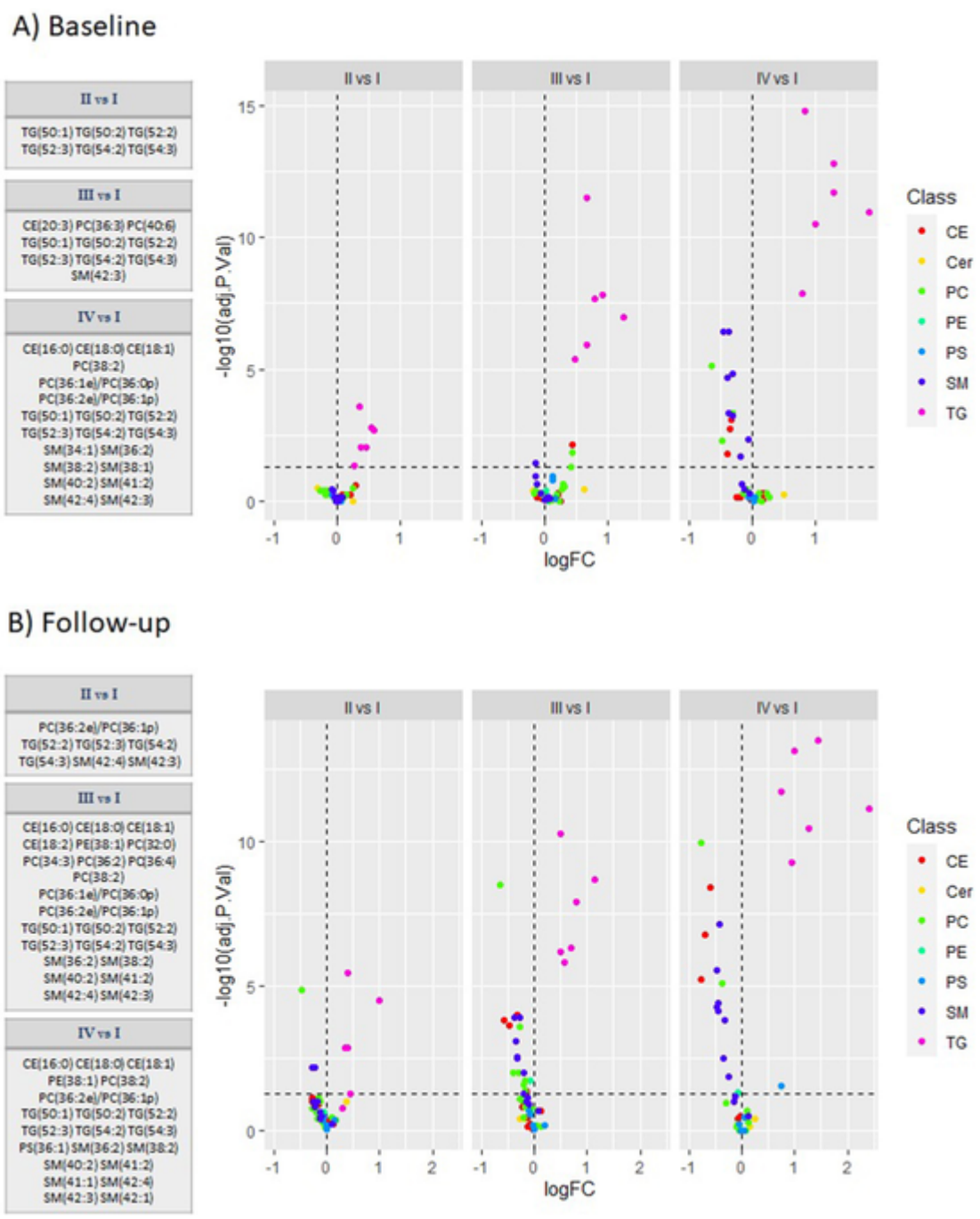


Fig. 2. Lipid profiling based on TG/HDL-C ratio groups at baseline (A) and follow-up (B). Volcano plots show lipids with altered plasma levels (adj. *p*-value threshold = 0.05) in groups with higher TG/HDL-C ratio as compared with the group with lower ratio (I Quartile). Axes x and y report respectively degrees of variation evaluated as log2 transformed fold change (logFC) and statistical significance as negative log10 transformed adjusted *p*-value (adj.P.Val). Lipid species are colored according to lipid class (CE, Cer, PC, PE, PS, SM, TG). The boxes on the left report in details lipid species, which were significantly altered in contrasts.

3.4. Partial correlations of lipidomic species with conventional lipid biomarkers

To investigate the correlation between lipidomic profile and traditional lipid biomarkers, partial correlation coefficients among the 51 lipid species and Total-C, LDL-C, HDL-C and triglycerides were assessed at baseline and follow-up and displayed as networks (Supplementary Figs. S2 and S3), which indicated that lipid species were mainly independent of conventional lipid biomarkers. At baseline, most of the 51 lipid species were related to each other in a single and interconnected network, whereas their correlations with conventional lipid biomarkers were feeble ($r < 0.25$), except for SM(36:2) and HDL-C ($r = 0.27$). At follow-up, the strong interconnection within most of 51 lipid species persisted, and only some weak relationship was present with conventional lipid biomarkers, including the negative relationship ($r = -0.33$) between LDL-C and PC(34:3), the negative relationship ($r = -0.30$, - 0.28, -0.29) between triglycerides and TG(50:1), CE (16:0), or CE(20:3) and, finally, the positive relationship ($r = 0.44$, 0.38) between triglycerides and TG(50:2), or TG(54:2). As expected, both at baseline and follow-up, strong relationships were found among all four conventional lipid biomarkers.

3.5. CTA score and targeted lipidomic profile

Lipidomic profiles at baseline and follow up were compared among groups with low, intermediate and high CTA risk score (Fig. 3). Baseline lipidomic profile was not statistically different comparing patients with intermediate or high risk score with respect to patients with low risk score (Fig. 3A). In contrast, at follow-up, 17 lipid species were significantly altered in the group with high as compared with the group with low risk score (Fig. 3B, Supplementary Table 2). In particular: plasma levels of 10 SMs [SM(36:2), SM(38:2), SM(38:1), SM(40:2), SM(40:1), SM(41:2), SM(41:1), SM(42:4), SM(42:3), SM(42:1)], CE(18:0) and 2 PCs [(PC(38:2), PC(36:2e)/PC(36:1p)] were decreased, with FC values between 0.7 and 0.8, while plasma levels of 4 TGs [TG(52:2), TG(52:3), TG(54:2), TG(54:3)] were increased, with FC values between 1.2 and 1.5.

Moreover, for some of altered SMs [SM(36:2), SM(38:2), SM(40:2), SM(42:4), SM(42:3)], and PC(38:2) plasma levels were significantly decreased, even if with lower FC, also in the group with intermediate as compared with the group with low risk score (Fig. 3B and Supplementary Table 2). Interestingly, 13 altered lipid species in the group with high risk score at follow-up were also altered in the groups with higher TG/HDL-C ratio both at baseline and follow-up (Fig. 4A). For these lipids, decreasing levels of SMs, PCs and CE(18:0) showed consistent differences with FC values around 0.6–0.8, while increasing levels of TGs were more evident in higher TG/HDL-C ratio groups than the high CTA score group.

Follow-up plasma levels of these 13 altered lipid species were compared according to the combinations of CTA score groups at baseline and follow-up used to assess CAD progression. Among them, CE(18:0), PC(38:2), PC(36:2e)/PC(36:1p, SM(36:2), SM(38:2), SM(40:2), SM

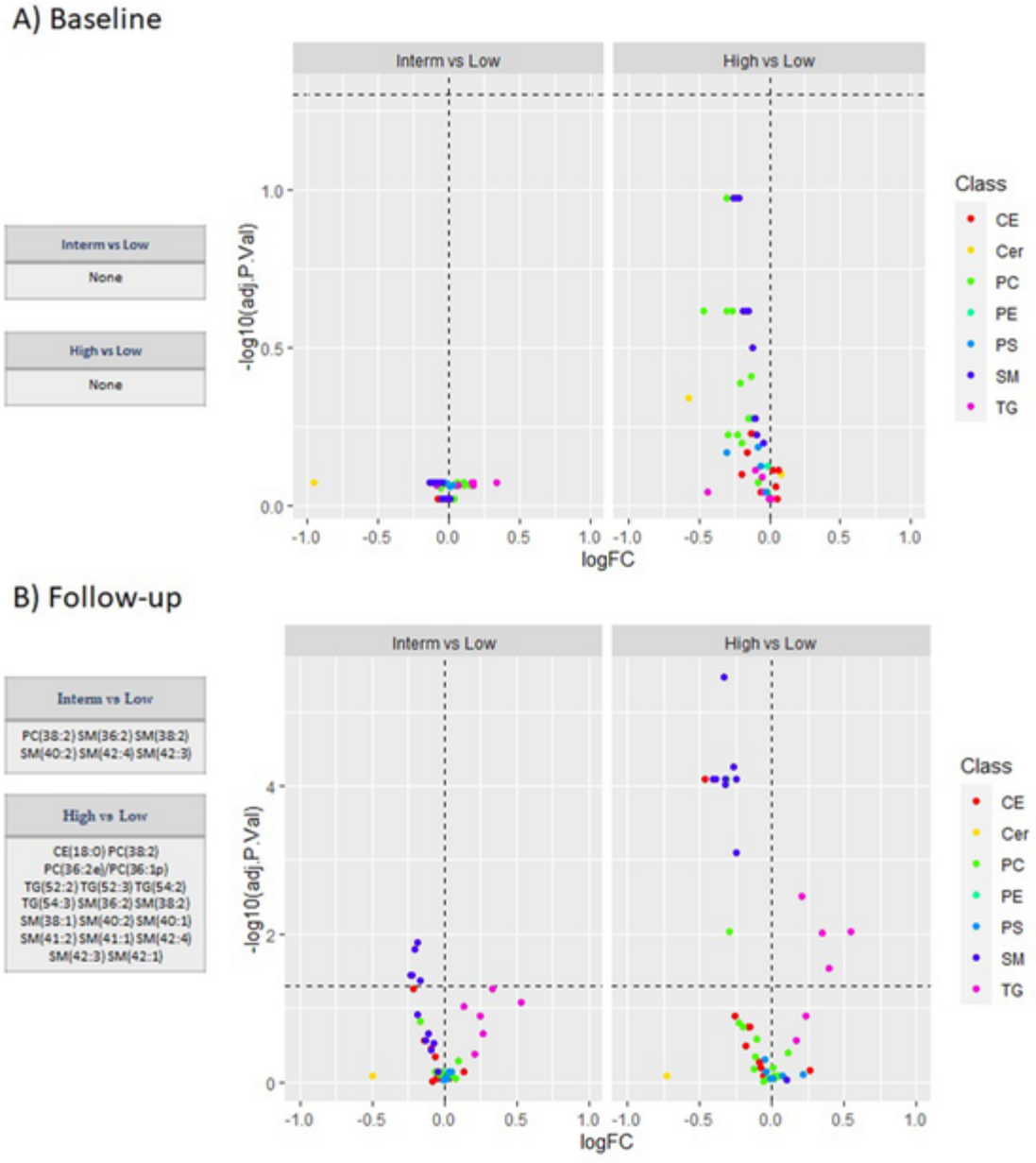
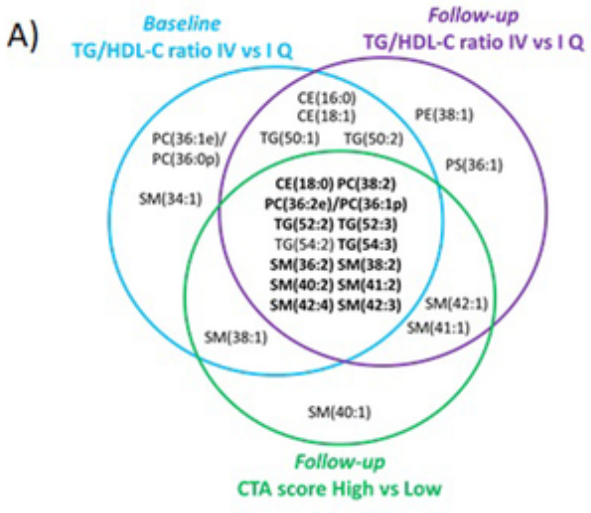


Fig. 3. Baseline lipid profile (A) and follow-up lipid profile (B) based on CTA score. Volcano plots showing lipids with altered plasma levels (adj. *p*-value threshold = 0.05) among groups of patients with Intermediate (CTA score 5–20) and High (CTA score >20) cardiovascular risk relative to those with Low cardiovascular risk (CTA score <5). Axes x and y report respectively degrees of variation evaluated as log2 transformed fold change (logFC) and statistical significance as negative log10 transformed adjusted *p*-value (adj.P.Val). Lipid species are colored according to lipid class (CE, Cer, PC, PE, PS, SM, TG). The boxes on the left report in details lipid species, which were significantly altered in contrasts.


A)
Baseline
TG/HDL-C ratio IV vs I Q
Follow-up
TG/HDL-C ratio IV vs I Q
Follow-up
CTA score High vs Low
CE(16:0)
CE(18:1)
PE(38:1)
PC(36:1e)/
PC(36:0p)
TG(50:1)
TG(50:2)
PS(36:1)
SM(34:1)
CE(18:0) PC(38:2)
PC(36:2e)/PC(36:1p)
TG(52:2) TG(52:3)
TG(54:2) TG(54:3)
SM(36:2) SM(38:2)
SM(40:2) SM(41:2)
SM(42:4) SM(42:3)
SM(42:1)
SM(41:1)
SM(38:1)
SM(40:1)

B)

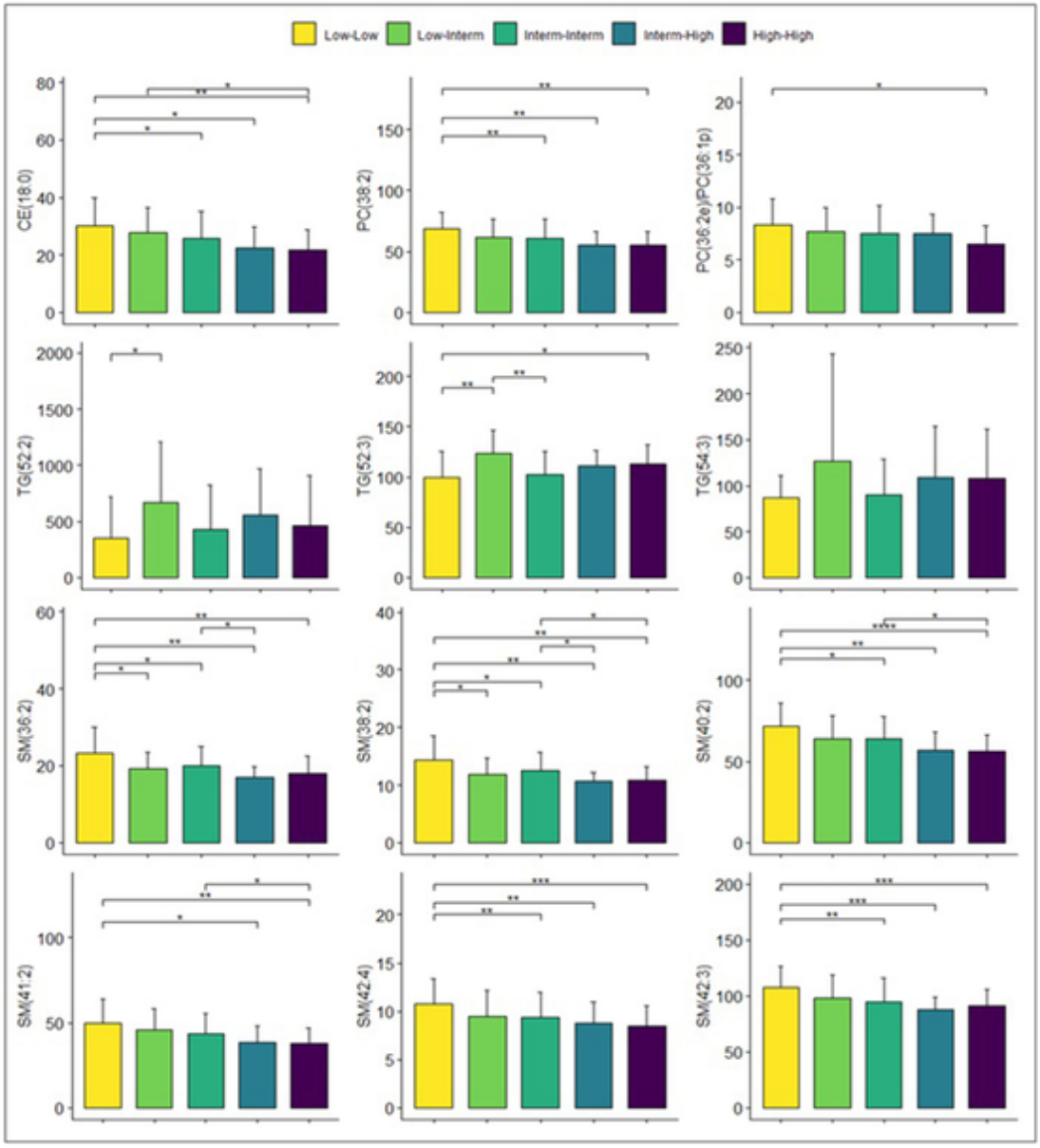

Low-Low
Low-Interm
Interm-Interm
Interm-High
High-High
CE(18:0)
PC(38:2)
PC(36:2e)/PC(36:1p)
TG(52:2)
TG(52:3)
TG(54:3)
SM(36:2)
SM(38:2)
SM(40:2)
SM(41:2)
SM(42:4)
SM(42:3)

Fig. 4. Identification of altered lipid species in common among groups defined by highest TG/HDL-C ratio at baseline and follow-up (IVQ) and highest CTA score at follow-up (CTA score >20) (A). A signature of 13 altered lipid species, included CE(18:0), PC(38:2), PC(36:2e)/PC(36:1p), TG(52:2), TG(52:3), TG(54:2), TG (54:3), SM(36:2), SM(38:2), SM(40:2), SM(41:2), SM(42:4), SM(42:3), was identified in common with patients with higher CAD risk. Bar plots reporting plasmatic concentration (μM) for 12 common altered lipid species across the categories assessing progression in combination with severity through changes from baseline to follow-up CTA score groups (B). Bars and error bars represent means and standard deviations (SD), respectively. Plasmatic levels of these lipids were investigated by pairwise comparisons using two-tailed Mann Whitney U-test and *p*-values were corrected using Benjamini-Hochberg procedure in order to minimize any type I error and thus occurrence of false positives. Only statistically significant differences are indicated as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

(41:2), SM(42:4) and SM(42:3) showed a significant decreasing trend along the CAD score group combinations (Fig. 4). Conversely, TG (52:2), TG(52:3) and TG(54:3) did not show a consistent trend, but increased particularly in the combination Low- Intermediate with respect to the others, and TG(52:2) increased in the two combinations showing disease progression (Low-Intermediate and Intermediate-High) (Fig. 4). Only TG(54:2) did not show any significant modification across pairs of CTA score groups (Supplementary Table 3).

4. Discussion

In the present study, a specific lipid species signature associated with high TG/HDL-C ratio was identified for the first time in plasma of stable patients with CCS. This specific lipid species profile, characterized by mass spectrometry analysis, was independent of the classical lipid biomarkers and was stable or even more evident after 6 years of follow-up despite the use of lipid-lowering medications and consequent reduction of Total-C, non-HDL-C and LDL-C levels. Very interestingly, in this selected population of stable patients under medical treatment, the coronary atherosclerotic risk, defined by CTA score, increased over time and the lipid signature associated with a high TG/HDL-C ratio was mirrored by a higher CTA risk score at follow-up.

In our population, the specific pattern of altered lipids associated with highest TG/HDL-C values (≥3.287) was characterized by increased levels of TG species, as expected, and reduced lipid species belonging to CE, PC and SM classes. These latter lipids are known to be part of HDL particles cargo [35]. It is well-known that HDL-C is an important coronary protective factor with extensive evidence of an inverse relationship between its serum levels and risk of cardiovascular disease [26,36] as well as with the presence and functional significance of obstructive CAD [26,27]. The beneficial role attributed to HDL-C is thought to reflect multiple cardioprotective properties of HDL particles, which primarily include the reverse cholesterol transport (RCT) from peripheral cells but may also involve antioxidative, anti-inflammatory, anti-apoptotic, anti-thrombotic, and anti-diabetic activities [37]. Nevertheless, it is not clear whether these protective functions are only related to the circulating levels of HDL-C. In fact, some hereditary conditions associated with lower HDL-C concentration are not consistently associated with higher cardiovascular risk and, on the contrary, genetic abnormalities or treatments able to increase circulating levels of this lipoprotein do not appear to confer additional prognostic benefits [38]. Indeed, current knowledge suggests that the biologic activity of HDLs may not depend solely on their concentration, but also on their quality. Alterations in various structural components of these lipoproteins lead to a state of dysfunction independent of their serum concentration [38]. In this context, the cholesterol efflux capacity of HDL appears a more effective feature in predicting the cardiovascular disease risk than HDL-C levels. PCs and SMs are reported to be the most abundant lipid components in HDL particles [35,39] and, according to their surface distribution, play a key role on cholesterol efflux capacity. Dysfunctional acute-phase HDL exhibit 25% less lipids per milligrams of protein, reflecting lower contents of SM and PC, and a substitution of 50% of CE with TG [40]. These lipid changes can alter antiatherogenic HDL assets, reducing their cholesterol efflux capacity and hindering RCT [38]. Moreover, high levels of TG species have an additional role in the functionality of HDLs. During the last phase of HDL particles metabolism, HDLs release CEs directly to the liver and steroidogenic tissues, where CEs are then metabolized into bile acid or neutral lipids [38]. The selective uptake of CEs is mediated by the scavenger receptor class B type I (SR-BI). TGs, like CEs, is a substrate for selective uptake by the SR-BI receptor. The selection of CEs or TGs for transfer is related directly to the TG/CE content of HDL particles [41]. The ability of HDLs to donate cholesterol for steroidogenesis or to promote RCT may be reduced when HDLs contain a high TG/CE ratio, as it occurs in hypertriglyceridemia and non insulin-dependent diabetes mellitus. Lastly, the alteration in TG/CE ratio in HDLs is also fundamental for their antioxidant activity and circulation [42], further confirming the role of elevated levels of TG in atherosclerosis.

Consistently with these mechanisms, the circulating lipid species signature identified by highest TG/HDL-C ratio values in our population included increase of TGs and decrease of CE, PC and SM classes with only a weak relationship with HDL-C plasma levels. Such condition of modified HDL-C lipids components was significantly associated with a higher CTA risk score at follow-up. These results suggest that the dysregulation of particular lipid species, mainly involving the sphingomyelin class, might specifically characterize a reduced functionality of HDLs, better reflecting atherogenesis than the overall reduction in HDL-C plasma levels.

More importantly, both this lipids pattern and coronary atherosclerotic burden assessed by CTA increased over time in our population of stable patients despite increased statin treatment and reduction in lipid biomarkers currently associated with atherogenesis. Despite the growing interest in identifying alteration in lipid metabolism involved in cardiovascular disease, the vast majority of lipidomics studies had focused on molecular markers that predict cardiovascular events [19], and only few studies focused on their exploitation as predictors of disease state. From these previous studies, consistently with the present findings, three HDL-associated phosphatidylcholines were identified as reduced in patients with stable CAD [43].

Additionally, we have recently demonstrated that in statin users CAD assessed by CTA is associated with specific circulating lipid species among the classes of sphingomyelins and phosphatidilethanolamines [20]. A common set of lipid biomarkers composed of 7 SMs and 3 PEs, which discriminates between high risk CAD patients and controls, was identified using three different CAD annotations considering CAD stenosis, extension and composition [20].

From a clinical point of view, the results of our analyses highlight the potentiality of measuring lipid species as new biomarkers of evolving atherosclerotic risk and to guide clinical management and target specific treatments in patients with CCS. In these patients, the identification of a specific condition of residual CAD risk not tackled by current treatments is a relevant clinical unmet need [12,44] and a potential target of new treatments [12,45].

We recognize some limitations of the present study. The major limitation is the observational design.

Furthermore, LDL-C was not determined directly but was calculated with the Friedewald formula. The study population was well characterized but was relatively small and included only patients at intermediate-low risk. The study was based on two European multicenter populations of patients enrolled in the EVINCI and ARTreat European FP7 projects because of clinical symptoms suggestive of obstructive CAD. Within these populations, only patients who were clinically stable over time and in whom coronary CTA was repeated by protocol at follow-up despite clinical stability were enrolled in the present study. Thus, it is

unknown whether our findings extend to a general population of asymptomatic subjects with similar risk factors or to higher risk populations with progressive symptoms or with cardiac dysfunction and heart failure.

In conclusion, results from this study extend the knowledge about the association of high TG and low HDL-C with CAD risk, unveiling by lipidomic analysis the potential involvement of underlying molecular pathways, not tackled by current treatments. In particular, in a European multicenter population of stable patients with CCS, highest TG/HDL-C ratio highlights a specific lipid species signature able to identify patients with higher CAD risk. The research of new molecular pathways has a pivotal importance in the fight against residual risk, and in development of new targeted nutritional, lifestyle and drug strategies beyond the established LDL-C lowering interventions [12,45].

Financial support

This research was funded by the European Commission in the H2020 program: Project SMARTool, "Simulation Modeling of coronary ARTery disease: a tool for clinical decision support— SMARTool" [GA number: 689068].

CRediT authorship contribution statement

Nicoletta Di Giorgi: Methodology, Data curation, Writing – original draft, preparation, Writing – review & editing. **Elena Michelucci:** Methodology, Writing – review & editing. **Jeff M. Smit:** Data curation, Writing – review & editing. **Arthur J.H.A. Scholte:** Investigation, Writing – review & editing. **Mohammed El Mahdiui:** Methodology, Writing – review & editing. **Juhani Knuuti:** Investigation, Writing – review & editing. **Ronny R. Buechel:** Investigation, Writing – review & editing. **Anna Teresinska:** Investigation, Writing – review & editing. **Maria N. Pizzi:** Investigation, Writing – review & editing. **Albert Roque:** Investigation, Writing – review & editing. **Rosa Poddighe:** Investigation, Writing – review & editing. **Oberdan Parodi:** Investigation, Writing – review & editing, Funding acquisition. **Gualtiero Pelosi:** Investigation, Data curation, Writing – review & editing. **Chiara Caselli:** Conceptualization, Methodology, Writing – original draft, preparation, Writing – review & editing, Supervision. **Danilo Neglia:** Conceptualization, Investigation, Writing – original draft, preparation, Writing – review & editing, Supervision. **Silvia Rocchiccioli:** Conceptualization, Writing – original draft, preparation, Writing – review & editing, Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank all SMARTool Clinical Investigators who make possible patient enrolment and availability of clinical data, CCTAs and blood samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.11.013.

References

[1] J. Knuuti, et al., 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes, Eur. Heart J. 41 (3) (2020) 407–477, https://doi.org/10.1093/eurheartj/ehz425.
[2] M. Murguía-Romero, et al., Plasma triglyceride/HDL-cholesterol ratio, insulin resistance, and cardiometabolic risk in young adults, J. Lipid Res. 54 (10) (2013) 2795–2799, https://doi.org/10.1194/jlr.M040584.
[3] M. Dobiášová, J. Frohlich, The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate inapob-lipoprotein-depleted plasma (FERHDL), Clin. Biochem. 34 (7) (2001) 583–588, https://doi.org/10.1016/S0009-9120(01)00263-6.
[4] V. Hanak, J. Munoz, J. Teague, A. Stanley, V. Bittner, Accuracy of the triglyceride to high-density lipoprotein cholesterol ratio for prediction of the low-density lipoprotein phenotype B, Am. J. Cardiol. 94 (2) (2004) 219–222, https://doi.org/10.1016/j.amjcard.2004.03.069.
[5] T. McLaughlin, et al., Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? Am. J. Cardiol. 96 (3) (2005) 399–404, https://doi.org/10.1016/j.amjcard.2005.03.085.
[6] L. Jia, et al., Relationship between total cholesterol/high-density lipoprotein cholesterol ratio, triglyceride/high-density lipoprotein cholesterol ratio, and high-density lipoprotein subclasses, Metabolism 55 (9) (2006) 1141–1148, https://doi.org/10.1016/j.metabol.2006.04.004.
[7] K. Hajian-Tilaki, B. Heidari, A. Bakhtiari, Triglyceride to high-density lipoprotein cholesterol and low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratios are predictors of cardiovascular risk in Iranian adults: evidence from a population-based cross-sectional study, Casp. J. Intern. Med. 11 (1) (2020) 53–61, https://doi.org/10.22088/cjim.11.1.53.
[8] J. Armato, G. Reaven, R. Ruby, Triglyceride/High-Density lipoprotein cholesterol concentration ratio identifies accentuated cardiometabolic risk, Endocr. Pract. 21 (5) (2015) 495–500, https://doi.org/10.4158/ep14479.or.
[9] J. Frohlich, M. Dobiášová, Fractional esterification rate of cholesterol and ratio of triglycerides to HDL-cholesterol are powerful predictors of positive findings on coronary angiography, Clin. Chem. 49 (11) (2003) 1873–1880, https://doi.org/10.1373/clinchem.2003.022558.
[10] H. Drexel, et al., Is atherosclerosis in diabetes and impaired fasting glucose driven by elevated LDL cholesterol or by decreased HDL cholesterol? Diabetes Care 28 (1) (2005) 101–107, https://doi.org/10.2337/diacare.28.1.101.
[11] Z. Chen, et al., Higher triglyceride to high-density lipoprotein cholesterol ratio increases cardiovascular risk: 10-year prospective study in a cohort of Chinese adults, J. Diabetes Investig. 11 (2) (2020) 475–481, https://doi.org/10.1111/jdi.13118.
[12] C. Caselli, et al., Elevated triglycerides and low HDL cholesterol predict coronary heart disease risk in patients with stable angina, Sci. Rep. (2021), https://doi.org/10.1038/s41598-021-00020-3.
[13] H.F. Avela, H. Sirén, Advances in lipidomics, Clin. Chim. Acta 510 (July) (2020) 123–141, https://doi.org/10.1016/j.cca.2020.06.049.
[14] J.M. Cheng, et al., Plasma concentrations of molecular lipid species in relation to coronary plaque characteristics and cardiovascular outcome: results of the ATHEROREMO-IVUS study, Atherosclerosis 243 (2) (2015) 560–566, https://doi.org/10.1016/j.atherosclerosis.2015.10.022.
[15] A.H. Ellims, G. Wong, J.M. Weir, P. Lew, P.J. Meikle, A.J. Taylor, Plasma lipidomic analysis predicts non-calcified coronary artery plaque in asymptomatic patients at intermediate risk of coronary artery disease, Eur. Heart J. Cardiovasc. Imag. 15 (8) (2014) 908–916, https://doi.org/10.1093/ehjci/jeu033.
[16] J.P. Karjalainen, et al., New evidence from plasma ceramides links apoE polymorphism to greater risk of coronary artery disease in Finnish adults, J. Lipid Res. 60 (9) (2019) 1622–1629, https://doi.org/10.1194/jlr.M092809.
[17] P.J. Meikle, et al., Plasma lipidomic analysis of stable and unstable coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 31 (11) (2011) 2723–2732, https://doi.org/10.1161/ATVBAHA.111.234096.
[18] C. Stegemann, et al., Lipidomics profiling and risk of cardiovascular disease in the prospective population-based bruneck study, Circulation 129 (18) (2014) 1821–1831, https://doi.org/10.1161/CIRCULATIONAHA.113.002500.
[19] S. Kohno, A.L. Keenan, J.M. Ntambi, M. Miyazaki, Lipidomic insight into cardiovascular diseases, Biochem. Biophys. Res. Commun. 504 (3) (2018) 590–595, https://doi.org/10.1016/j.bbrc.2018.04.106.
[20] E. Michelucci, et al., Lipid biomarkers in statin users with coronary artery disease annotated by coronary computed tomography angiography, Sci. Rep. (2021) 1–8, https://doi.org/10.1038/s41598-021-92339-0.
[21] A.I. Sakellarios, et al., SMARTool: a tool for clinical decision support for the management of patients with coronary artery disease based on modeling of atherosclerotic plaque process, Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS (2017) 96–99, https://doi.org/10.1109/EMBC.2017.8036771.
[22] J.M. Smit, et al., Impact of clinical characteristics and statins on coronary plaque progression by serial computed tomography angiography, Circ. Cardiovasc. Imag. (March) (2020) 1–9, https://doi.org/10.1161/CIRCIMAGING.119.009750.
[23] A.I. Sakellarios, et al., "Non-Invasive prediction of site-specific coronary atherosclerotic plaque progression using lipidomics, blood flow, and LDL transport modeling, Appl. Sci. 11 (5) (2021).
[24] S. Sbrana, et al., Blood monocyte phenotype fingerprint of stable coronary artery disease: a cross- sectional substudy of SMARTool clinical trial, BioMed Res. Int. 2020 (2020), https://doi.org/10.1155/2020/8748934.
[25] M. El Mahdiui, et al., Sex differences in coronary plaque changes assessed by serial computed tomography angiography, Int. J. Cardiovasc. Imag. (2021),

0123456789, https://doi.org/10.1007/s10554-021-02204-4.
[26] C. Caselli, et al., HDL cholesterol, leptin and interleukin-6 predict high risk coronary anatomy assessed by CT angiography in patients with stable chest pain, Atherosclerosis 241 (1) (2015) 55–61, https://doi.org/10.1016/j.atherosclerosis.2015.04.811.
[27] C. Caselli, et al., A new integrated clinical-biohumoral model to Predict Functionally significant coronary artery disease inPatients with chronic chest pain, Can. J. Cardiol. 31 (6) (2015) 709–716, https://doi.org/10.1016/j.cjca.2015.01.035.
[28] C. Caselli, et al., Effect of coronary atherosclerosis and myocardial ischemia on plasma levels of high-sensitivity troponin T and NT-proBNP in patients with stable Angina, Arterioscler. Thromb. Vasc. Biol. 36 (4) (2016) 757–764, https://doi.org/10.1161/ATVBAHA.115.306818.
[29] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentrationof low-density lipoprotein cholesterolin plasma, without useof the preparative ultracentrifuge, J. Chem. Inf. Model. 53 (9) (2013) 1689–1699.
[30] B.R. Wg Austen, J.E. Edwards, R.L. Frye, G.G. Gensini, V.L. Gott, L.S. Griffith, D.C. McGoon, M.L. Murphy, Aha committee report: a reporting system on patients evaluated for coronary artery disease report of the ad hoc committee for grading of coronary artery, Circulation 51 (4) (1975) 5–40.
[31] R.C. Cury, et al., Coronary artery disease - reporting and data system (CAD-RADS): an expert consensus document of SCCT, ACR and NASCI: endorsed by the ACC, JACC Cardiovasc. Imag. 9 (9) (2016) 1099–1113, https://doi.org/10.1016/j.jcmg.2016.05.005.
[32] M.A. De Graaf, et al., Automatic quantification and characterization of coronary atherosclerosis with computed tomography coronary angiography: cross-correlation with intravascular ultrasound virtual histology, Int. J. Cardiovasc. Imag. 29 (5) (2013) 1177–1190, https://doi.org/10.1007/s10554-013-0194-x.
[33] A.R. van Rosendael, et al., Superior risk stratification with coronary computed tomography angiography using a comprehensive atherosclerotic risk score, JACC Cardiovasc. Imag. 12 (10) (2019) 1987–1997, https://doi.org/10.1016/j.jcmg.2018.10.024.
[34] J. Folch, M. Lees, G.S. Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1) (1957) 497–509.
[35] R. Martínez-Beamonte, J.M. Lou-Bonafonte, M.V. Martínez-Gracia, J. Osada, Sphingomyelin in high-density lipoproteins: structural role and biological function, Int. J. Mol. Sci. 14 (4) (2013) 7716–7741, https://doi.org/10.3390/ijms14047716.
[36] P.W.F. Wilson, R.D. Abbott, W.P. Castelli, High density lipoprotein cholesterol and mortality. The Framingham heart study, Arteriosclerosis 8 (6) (1988) 737–741, https://doi.org/10.1161/01.atv.8.6.737.
[37] L. Camont, M.J. Chapman, A. Kontush, Biological activities of HDL subpopulations and their relevance to cardiovascular disease, Trends Mol. Med. 17 (10) (2011) 594–603, https://doi.org/10.1016/j.molmed.2011.05.013.
[38] J. Salazar, L.C. Olivar, E. Ramos, M. Chávez-Castillo, J. Rojas, V. Bermúdez, Dysfunctional high-density lipoprotein: an innovative target for proteomics and lipidomics, Cholesterol 2015 (2015), https://doi.org/10.1155/2015/296417.
[39] P. Wiesner, K. Leidl, A. Boettcher, G. Schmitz, G. Liebisch, Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry, J. Lipid Res. 50 (3) (2009) 574–585, https://doi.org/10.1194/jlr.D800028-JLR200.
[40] W. Pruzanski, E. Stefanski, F.C. De Beer, M.C. De Beer, A. Ravandi, A. Kuksis, Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins, J. Lipid Res. 41 (7) (2000) 1035–1047, https://doi.org/10.1016/S0022-2275(20)32007-1.
[41] D.J. Greene, J.W. Skeggs, R.E. Morton, Elevated triglyceride content diminishes the capacity of high density lipoprotein to deliver cholesteryl esters via the scavenger receptor class B type I (SR-BI), J. Biol. Chem. 276 (7) (2001) 4804–4811, https://doi.org/10.1074/jbc.M008725200.
[42] A. Kontush, E.C. De Faria, S. Chantepie, M.J. Chapman, A normotriglyceridemic, low HDL- cholesterol phenotype is characterised by elevated oxidative stress and HDL particles with attenuated antioxidative activity, Atherosclerosis 182 (2) (2005) 277–285, https://doi.org/10.1016/j.atherosclerosis.2005.03.001.
[43] I. Sutter, et al., Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL, Atherosclerosis 241 (2) (2015) 539–546, https://doi.org/10.1016/j.atherosclerosis.2015.05.037.
[44] D.S. Dhindsa, P.B. Sandesara, M.D. Shapiro, N.D. Wong, The evolving understanding and approach to residual cardiovascular risk management, Front. Cardiovasc. Med. 7 (May) (2020), https://doi.org/10.3389/fcvm.2020.00088.
[45] P. Mourikis, et al., Lipid lowering therapy in cardiovascular disease: from myth to molecular reality, Pharmacol. Ther. 213 (2020) 107592, https://doi.org/10.1016/j.pharmthera.2020.107592.