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Plasma levels of apolipoproteins C-III, A-IV, and E are independently associated with stable atherosclerotic cardiovascular disease☆

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HIGHLIGHTS

- Concentrations of apos A-II, B-100, C-I, and E were decreased under statin therapy.
- Strongest associations with atherosclerosis were seen for VLDL-associated apos.
- ApoC-III and apoE were independently associated with carotid artery plaque.
- ApoA-IV was inversely correlated with CAD only under fasting conditions.
- Ratios of apos did not improve atherosclerosis risk prediction.

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ABSTRACT

Background and aims: Dyslipidemia is a major risk factor for atherosclerotic cardiovascular disease (ASCVD). As key regulators of lipoprotein metabolism, apolipoproteins (apos) are discussed as vascular risk factors. This study aimed to analyze associations of major plasma apos with coronary artery disease (CAD), peripheral artery disease (PAD) and carotid artery plaque (CAP) to elucidate their diagnostic potential in risk assessment.
Methods: ApoA-I, apoA-II, apoA-IV, apoB-100, apoC-I, apoC-III, apoE, and apoJ were simultaneously quantified in 3 μL EDTA-plasma by LC-MS/MS in a case-control subgroup of the Leipziger LIFE-Heart Study (N = 911). Confounder analysis with demographic, clinical covariates and serum lipids, cardiac, inflammatory, and hepatic markers were performed. Apos were associated with CAD, CAP, and PAD in a multivariate regression model.
Results: Fasting and statin therapy showed strongest effects on apo concentrations. Inverse correlations of HDL-related apos A-I, A-II, A-IV, and C-I were observed for troponin T and interleukin 6. Concentrations of apos A-II, B-100, C-I, and E were decreased under statin therapy. After adjustment for influencing factors and related lipids, only apoB-100 (odds ratio per one SD [OR], 1.39; 95% confidence interval [CI], 1.05–1.84) was independently associated with CAD while apoA-IV (OR, 0.74; 95% CI 0.58–0.95) indicated PAD. ApoB-100 (OR, 1.55; 95% CI, 1.18–2.04), apoC-III (OR, 1.30; 95% CI, 1.06–1.58), and apoE (OR, 1.34; 95% CI, 1.13–1.58) were associated with CAP.
Conclusions: Triglyceride rich lipoproteins (TRLs) associated apos A-IV, B-100, C-III, and E are independently associated with stable ASCVD, providing further evidence for a potential role of TRLs in atherogenesis.

1. Introduction

Lipoproteins are micelle-like particles transporting neutral lipids in blood and extracellular fluid. Metabolic functions of lipoproteins are mostly controlled by interactions of apolipoproteins (apo) presented on the particles' surfaces with cell membrane receptors, enzymes, and lipid transfer proteins. Their central role in lipid metabolism links apos to diagnosis and treatment of dyslipidemias and atherosclerotic

☆ Dedicated to Prof. Dietrich Seidel, previous Editor-in-Chief of *Atherosclerosis*, on the occasion of his 80th birthday.

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cardiovascular disease (ASCVD). In this regard, apoA-I and apoB were proposed as markers superior to HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C) for the assessment of cardiovascular risk, advocating the relevance of quantifying apos in the clinical routine [1,2]. Besides, apoC-III, a potent modulator of lipoprotein lipase (LPL), was identified as risk marker of incident coronary artery disease (CAD) [3], which is in line with current evidence on a causal role of triglyceride-rich lipoproteins (TRL) and TRL remnants in ASCVD [4–6]. ApoE isoforms [7], especially apoE2 and apoE4, as well as apoA-IV, which was found to be inversely correlated with CAD [8–10], are further biomarkers of ASCVD.

Despite the potential utility of plasma apos for the cardiovascular risk assessment, their application was limited due to a lack of standardized clinical routine laboratory applications, with the exception of apoB and apoA-I. In recent years, targeted proteomics assays have been developed, which enable a reliable and simultaneous analysis of multiple apos based on liquid chromatography combined with mass spectrometry (LC-MS/MS) [11–15]. Though, only few studies have investigated plasma apos in ASCVD, focusing on associations with incident acute coronary syndromes and their predictive value in population-based studies or small patient groups [3,16,17]. In that regard, correlations of apoC-II, apoC-III, and apoE with incident ASCVD events were recently demonstrated by Pechlaner et al. in a very first application of a multiplexed apo analysis [16]. For a deeper understanding of the role of apos in ASCVD, the present study aims to investigate associations of apos A-I, A-II, A-IV, B-100, C-I, C-III, E, and J with peripheral artery disease (PAD) defined by ankle-brachial index (ABI), carotid artery plaque (CAP), assessed by ultrasound, and angiographically proven CAD.

2. Materials and methods

2.1. Study design and participants

The LIFE-Heart Study is an observational study of patients recruited at the Leipzig Heart Center, Germany. The study was approved by the Ethics Committee of the Faculty of Medicine of Leipzig University, Germany (Reg. No 276–2005) and is registered at ClinicalTrials.gov (NCT00497887). Written informed consent was obtained from all participants included in the study. Patients with suspected stable CAD or myocardial infarction were recruited. The study design and a detailed description of patients can be found elsewhere [18,19]. For the present case-control subsample, individuals with myocardial infarction were excluded. Subsets of patients with stenosis ≥50% in one or more major coronary arteries (n = 531), confirmed by catheterization, and of patients with angiographically normal coronary arteries (n = 458) were included in the following analysis. CAP was assessed by ultrasound and was defined as a focal structure protruding ≥0.5 mm into the lumen of the common part or bulb of the right and left carotid artery, respectively, or reaching a thickness ≥50% of the surrounding intima [20]. PAD diagnosis was based on the Doppler sonographic measurement of ABI. An ABI < 0.9 in one leg or anamnestic information was used to classify PAD. CAD cases were diagnosed with a stenosis ≥50% in at least one major coronary vessel. Subjects were considered at fasting, if blood was drawn at least 8 h after food intake. Individuals with renal impairment (serum creatinine concentration > 132 µmol/L) or incomplete medical history documentation were excluded retrospectively. Finally, 911 subjects were included in the statistical evaluation.

2.2. Laboratory analyses

Samples were processed using a standardized pre-analytical protocol as previously described [18]. LC-MS/MS analysis of apos was performed according to a previously published method developed in our laboratory [21]. Laboratory measurements, including troponin T, N-terminal brain natriuretic peptide (NT-proBNP), interleukin 6 (IL-6), C-reactive protein (CRP), serum creatinine, glucose, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (g-GT), and HbA1c were performed on the same day of blood sampling using an automated Roche Cobas 6000 Clinical Chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Triglycerides (TG), total cholesterol, LDL-C, and HDL-C were determined by homogeneous enzymatic colorimetric assays (Roche Diagnostics). Non-HDL-C was calculated by subtraction of HDL-C from total cholesterol.

2.3. Statistical analyses

Statistical processing was performed with IBM® SPSS® Statistics, version 20 (Armonk, NY, USA). p values were two-sided and alpha < 0.05 was used for hypothesis testing. Baseline characteristics were reported as median (interquartile range) or percentage unless otherwise stated. Basic group comparisons were performed using Mann-Whitney U test and Pearson's chi-square test. Apos and lipid parameters were log-transformed to approximate normal distributions. Agglomerative hierarchical cluster analysis on the basis of average linkage was used to cluster apos and lipid variables. Proximity measures were based on Pearson correlation. Correlations of apos and lipids with other diagnostic parameters were analyzed according to Spearman. Factors of influence on apo concentrations were assessed using linear multivariable regression analysis. Clinical covariates including sex, age, body mass index (BMI), fasting status, smoking, statin medication, and diabetes mellitus were investigated as predictors of apo concentrations. Resulting regression coefficients are presented as antilogarithms. Associations of apos and CAD, CAP, and PAD were evaluated using logistic regression analysis considering identified influencing factors and related lipid parameters.

3. Results

3.1. Baseline characteristics

Demographic and clinical characteristics of cases with obstructive CAD, CAP or PAD and respective controls are summarized in Table 1. Angiographic examination of the investigated subset revealed that most CAD cases (43%) suffered from an obstructive stenotic lesion in three coronary vessels. In addition, 73% and 22% of patients with stable CAD were also diagnosed with CAP or PAD, respectively. The prevalence of traditional cardiovascular risk factors was higher in CAD cases than in respective controls. In detail, CAD patients were mainly of male gender, older, showed a higher rate of diabetes mellitus and smoked more frequently in comparison to controls. In CAP and PAD, risk factors like higher age and diabetes were also more prevalent in cases than in controls.

Total cholesterol levels did not differ between ASCVD cases and controls. By contrast, concentrations of HDL-C were lower in the groups of cases while LDL-C was significantly increased in CAD and CAP cases, but not in patients suffering from PAD. In CAD patients, TG plasma concentrations were elevated compared to respective controls. Additionally, plasma levels of troponin T, NT-proBNP, IL-6, and CRP as well as those of creatinine, glucose, and HbA1c were higher in ASCVD subjects (Table 1).

3.2. Correlation of apo levels, plasma lipids and other blood parameters

In a correlation analysis of plasma apos and standard lipid parameters, we found two clusters of highly associated variables that were formed by apoB-100, LDL-C, and non-HDL-C as well as apos C-I, C-III, E, and TG (Fig. 1). ApoC-I, which is present on TRLs like VLDL, was also correlated to a third cluster of interrelated variables which comprised apoA-I, apoA-II, and HDL-C. Overall, strongest correlations of apos were

Table 1
Baseline characteristics of the case-control subgroup of the LIFE-Heart Study (N = 911).

	Overall	Stable coronary artery disease			Carotid artery plaque			Peripheral artery disease		
		Controls	Cases	*p* value	Controls	Cases	*p* value	Controls	Cases	*p* value
N	911	442	469		444	462		764	109	
Men, %	62.6	49.1	75.3	< 0.001	53.2	71.6	< 0.001	62.2	68.8	0.180
Age, yrs	63 (54–70)	59 (51–68)	66 (56–72)	< 0.001	59 (51–69)	66 (56–71)	< 0.001	61 (53–70)	68 (58–74)	< 0.001
Body mass index, kg/m^2	29.1 (26.3–32.6)	29.1 (26.3–32.8)	29.0 (26.3–32.4)	0.674	29.2 (26.0–33.0)	29.0 (26.4–32.3)	0.447	29.0 (26.3–32.5)	29.2 (25.6–32.5)	0.579
Diabetes mellitus, %	28.6	21.0	35.8	< 0.001	21.6	35.3	< 0.001	26.2	46.8	< 0.001
Current smoker, %	16.0	12.0	19.8	0.001	14.9	17.1	0.359	14.0	30.3	< 0.001
Statin therapy %	33.7	25.1	41.8	< 0.001	26.8	39.8	< 0.001	31.9	46.8	0.002
Fasting, %	31.5	27.1	35.6	0.006	30.9	31.8	0.755	29.8	37.6	0.100
CAD (n [%])	469 (51.5)				121 (27.3)	343 (74.2)	< 0.001	347 (45.4)	103 (94.5)	< 0.001
Single-vessel disease	137 (15.0)		137 (29.2)		44 (9.9)	93 (20.1)		116 (15.2)	15 (13.8)	
Double-vessel disease	132 (14.5)		132 (28.1)		42 (9.5)	90 (19.5)		98 (12.8)	27 (24.8)	
Triple-vessel disease	200 (22.0)		200 (42.6)		35 (7.9)	160 (34.6)		133 (17.4)	61 (56.0)	
CAP (n [%])	462 (50.7)	119 (26.9)	343 (73.1)	< 0.001				350 (45.8)	92 (84.4)	< 0.001
PAD (n [%])	109 (12.0)	6 (1.4)	103 (22.0)	< 0.001	15 (3.4)	92 (19.9)	< 0.001			
Total cholesterol, mmol/L	5.34 (4.64–6.21)	5.31 (4.73–5.99)	5.41 (4.51–6.46)	0.125	5.30 (4.60–6.11)	5.41 (4.66–6.41)	0.096	5.40 (4.64–6.24)	5.25 (4.52–6.11)	0.353
HDL cholesterol, mmol/L	1.27 (1.06–1.53)	1.38 (1.14–1.62)	1.19 (1.01–1.45)	< 0.001	1.34 (1.11–1.61)	1.23 (1.02–1.48)	< 0.001	1.29 (1.07–1.54)	1.15 (0.96–1.50)	0.001
LDL cholesterol, mmol/L	3.30 (2.63–4.04)	3.20 (2.6–3.86)	3.46 (2.67–4.33)	0.001	3.18 (2.54–3.93)	3.46 (2.70–4.24)	0.003	3.31 (2.64–4.04)	3.23 (2.56–4.08)	0.679
Triglycerides, mmol/L	1.67 (1.20–2.42)	1.62 (1.09–2.37)	1.75 (1.25–2.48)	0.016	1.64 (1.13–2.38)	1.72 (1.25–2.48)	0.099	1.66 (1.21–2.42)	1.78 (1.17–2.43)	0.841
Lipoprotein a, g/L	0.2 (0.1–0.4)	0.2 (0.1–0.4)	0.2 (0.1–0.6)	0.004	0.2 (0.1–0.4)	0.2 (0.1–0.5)	0.805	0.2 (0.1–0.4)	0.2 (0.1–0.6)	0.521
Troponin T, pg/mL	9.2 (5.3–16.4)	6.4 (4.1–10.4)	12.8 (7.8–25.1)	< 0.001	7.2 (4.4–13)	10.5 (7–20.3)	< 0.001	8.5 (5.1–14.5)	16.3 (10–35.1)	< 0.001
NT-proBNP, pg/mL	145 (62–418)	102 (45–239)	240 (85–638)	< 0.001	107 (51–328)	204 (78–564)	< 0.001	128 (53–354)	448 (174–1135)	< 0.001
High-sensitive CRP, mg/L	2.3 (1.1–5.1)	1.9 (1.0–3.9)	2.8 (1.3–6.0)	< 0.001	2.0 (1.0–4.4)	2.7 (1.3–5.7)	< 0.001	2.2 (1.1–4.5)	4.3 (2.1–10.7)	< 0.001
Interleukin 6, pg/mL	2.5 (1.5–4.5)	1.9 (1.5–3.4)	3.1 (1.7–6.3)	< 0.001	2.2 (1.5–3.9)	2.8 (1.6–5.6)	< 0.001	2.3 (1.5–4.0)	5.1 (2.8–8.7)	< 0.001
ALT, µkat/L	0.45 (0.32–0.65)	0.43 (0.31–0.67)	0.46 (0.33–0.65)	0.355	0.45 (0.32–0.68)	0.45 (0.33–0.63)	0.792	0.45 (0.33–0.67)	0.37 (0.29–0.62)	< 0.001
AST, µkat/L	0.45 (0.37–0.57)	0.45 (0.37–0.55)	0.46 (0.38–0.59)	0.073	0.45 (0.37–0.57)	0.45 (0.37–0.58)	0.522	0.45 (0.37–0.57)	0.46 (0.36–0.62)	0.766
ALP, µkat/L	1.18 (0.98–1.42)	1.16 (0.97–1.42)	1.2 (1.01–1.41)	0.146	1.17 (0.98–1.43)	1.19 (0.98–1.41)	0.749	1.16 (0.97–1.41)	1.26 (1.07–1.43)	0.05
g-GT, µkat/L	0.56 (0.37–0.94)	0.49 (0.34–0.87)	0.62 (0.41–1.04)	< 0.001	0.51 (0.35–0.87)	0.61 (0.4–1.05)	0.001	0.55 (0.38–0.92)	0.6 (0.37–1.07)	0.402
Bilirubin, µmol/L	9.9 (7.4–13.3)	9.9 (7.1–13.1)	10 (7.7–13.4)	0.124	10 (7.3–13.1)	9.8 (7.4–13.4)	0.894	9.9 (7.3–13.1)	10 (7.7–13.7)	0.544
Creatinine, µmol/L	77 (66–89)	73 (64–84)	80 (70–92)	< 0.001	74 (64–87)	79 (68–91)	< 0.001	76 (66–88)	80 (67–93)	0.035
Glucose, mmol/L	6.03 (5.34–7.4)	5.78 (5.23–6.79)	6.34 (5.49–7.86)	< 0.001	5.88 (5.26–6.86)	6.22 (5.42–7.87)	< 0.001	6.01 (5.34–7.19)	6.82 (5.42–9.86)	0.001
HbA_{1c}, %	5.7 (5.4–6.1)	5.6 (5.4–6.0)	5.8 (5.5–6.4)	< 0.001	5.7 (5.4–6.0)	5.8 (5.5–6.4)	< 0.001	5.7 (5.4–6.1)	6.0 (5.5–6.7)	< 0.001
TSH, mU/L	1.2 (0.7–1.8)	1.2 (0.8–1.8)	1.1 (0.7–1.7)	0.184	1.2 (0.8–1.9)	1.1 (0.7–1.7)	0.042	1.2 (0.8–1.8)	1.2 (0.6–1.8)	0.524

Values are presented as median (interquartile range) unless otherwise stated. *p* values of continuous and dichotomous variables were calculated using Mann-Whitney *U* test and χ^2 test, respectively.
ALP indicates alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CAD, coronary artery disease; CAP, carotid artery plaque; g-GT, gamma-glutamyltransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAD, peripheral artery disease; TSH, thyroid-stimulating hormone.

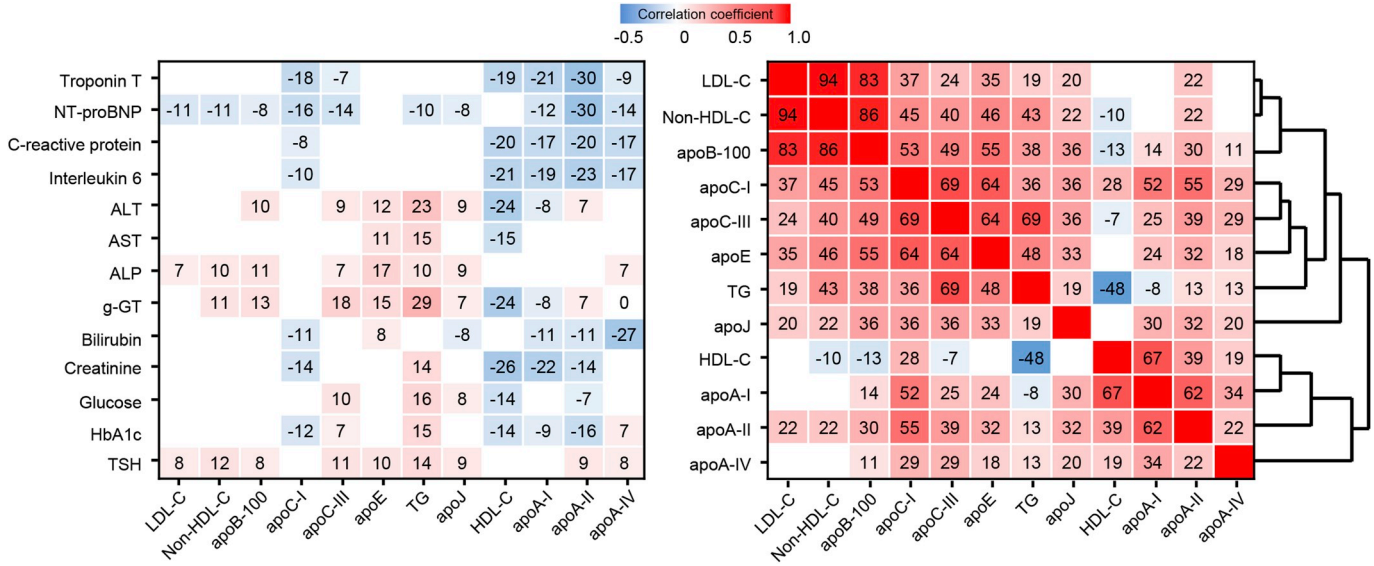

Fig. 1. Correlations of apolipoproteins in the LIFE-Heart Study.
(Left panel) Correlations of apolipoproteins and lipids with parameters of inflammation, heart failure, carbohydrate metabolism as well as liver and kidney function (Spearman correlation). (Right panel) Interrelationships of apolipoproteins and lipids (Pearson correlation). Square color indicates the magnitude of correlation, whose first two decimal digits are given as text. Only significant correlations are shown. Arrangement of variables was performed according to similarity as depicted by the dendrogram. ALP, alkaline phosphatase; ALT, alanine transaminase; apo, apolipoprotein; AST, aspartate transaminase; g-GT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; TG, triglycerides.

Table 2
Factors of influence on apolipoprotein and lipid plasma concentrations in the case-control subgroup of the LIFE-Heart Study (N = 911).

	Age		Sex (male gender)		BMI		Diabetes (non-diabetic)		Fasting (non-fasted)		Statin therapy (unmedicated)		Smoking (non-smoking)	
	β	*p* value	β	*p* value	β	*p* value	β	*p* value	β	*p* value	β	*p* value	β	*p* value
apoA-I	0.999	0.309	**1.098**	**< 0.001**	**0.995**	**0.002**	**0.968**	**0.043**	**0.961**	**0.008**	0.982	0.207	0.976	0.219
apoA-II	**0.999**	**< 0.001**	1.013	0.393	**0.997**	**0.020**	**0.950**	**0.002**	**0.970**	**0.049**	**0.959**	**0.005**	0.986	0.474
apoA-IV	1.002	0.206	1.024	0.369	0.997	0.250	**1.071**	**0.019**	**0.800**	**< 0.001**	0.951	0.059	0.977	0.510
apoB-100	1.000	0.764	**0.956**	**0.028**	**1.006**	**0.003**	0.972	0.210	0.998	0.930	**0.844**	**< 0.001**	**1.073**	**0.013**
apoC-I	**0.998**	**0.036**	**1.059**	**0.002**	1.001	0.638	**0.958**	**0.035**	**0.938**	**0.001**	**0.909**	**< 0.001**	1.021	0.409
apoC-III	0.999	0.200	0.987	0.533	**1.008**	**< 0.001**	1.033	0.168	**0.927**	**0.001**	0.981	0.362	1.004	0.885
apoE	1.001	0.216	1.041	0.096	**1.008**	**0.001**	1.006	0.830	**0.951**	**0.045**	**0.868**	**< 0.001**	1.061	0.077
apoJ	**0.998**	**0.002**	1.004	0.765	1.001	0.418	**1.032**	**0.036**	0.974	0.058	0.983	0.203	1.026	0.170
HDL-C	1.001	0.078	**1.226**	**< 0.001**	**0.988**	**< 0.001**	**0.938**	**0.001**	0.975	0.168	0.973	0.127	**0.927**	**0.001**
LDL-C	1.000	0.628	1.003	0.889	**1.005**	**0.035**	**0.940**	**0.007**	1.030	0.181	**0.779**	**< 0.001**	1.023	0.421
non-HDL-C	0.999	0.223	0.973	0.150	**1.007**	**0.001**	0.967	0.106	0.995	0.813	**0.809**	**< 0.001**	1.039	0.141
TG	0.997	0.098	**0.809**	**< 0.001**	**1.023**	**< 0.001**	**1.112**	**0.004**	**0.774**	**< 0.001**	0.965	0.295	**1.115**	**0.017**
Total cholesterol	0.999	0.444	**1.035**	**0.015**	1.002	0.184	**0.962**	**0.015**	0.991	0.558	**0.849**	**< 0.001**	1.012	0.531

Regression coefficients identified in a multivariable linear regression are given as antilogarithms and correspond to a one-unit change. Reference categories are given in parenthesis. Significant influences are written in bold.
Apo, apolipoprotein; β, regression coefficient; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglycerides.

found for apoB-100 with non-HDL-C (Pearson's R, $r_P = 0.86$) and LDL-C ($r_P = 0.83$); apoC-III with TG ($r_P = 0.69$) and apoC-I ($r_P = 0.69$) as well as for apoA-I with HDL-C ($r_P = 0.67$). ApoA-IV and apoJ showed modest correlations with most other lipids and apos.

Various bivariate correlations between apos and cardiac, hepatic, renal as well as inflammatory markers are summarized in Fig. 1. Inverse correlations with IL-6 and NT-proBNP were observed for apos A-I, A-II, A-IV, and C-I. Thereby, strongest associations were determined for apoA-II with troponin T (Spearman's R, $r_S = -0.30$) and NT-proBNP ($r_S = -0.30$), respectively. In contrast, positive associations with ALT, ALP, g-GT were observed for apos B-100, C-III, E, and J.

3.3. Influencing factors on plasma Apo levels

We investigated influences of the cardiovascular risk factors age, sex, BMI, diabetes, and active smoking and of other potential confounders such as statin medication and fasting status on plasma apo levels (Table 2). Higher plasma concentrations of apoA-I (+10%), apoC-I (+6%), and HDL-C (+23%) were seen in females compared to males while opposite effects were found for apoB-100 (−4%) and TG (−19%). Increasing age or BMI had only minor impact on plasma apos. Active smoking solely affected apoB-100 (+7%), HDL-C (−7%), and TG (+12%). In contrast, diabetes altered plasma levels of most apos and lipids except for apoB-100, apoC-III, apoE, and non-HDL-C. Levels

Table 3
Apolipoprotein distributions between cases and controls in the 3 ASCVD models.

	Overall	Stable coronary artery disease			Carotid artery plaque			Peripheral artery disease		
		Controls	Cases	*p* value	Controls	Cases	*p* value	Controls	Cases	*p* value
N	911	442	469		444	462		764	109	
apoA-I, μmol/L	57.8 (49.9–66.5)	59.9 (52.0–68.0)	55.9 (48.5–63.8)	< 0.001	58.9 (51.1–67.9)	56.9 (48.6–64.8)	0.001	58.5 (50.4–67.0)	55.0 (48.2–62.7)	0.011
apoA-II, μmol/L	47.3 (41.0–54.7)	48.5 (42.3–56.0)	46.4 (40.1–53.6)	0.001	48.5 (42.3–56.1)	46.3 (40.0–52.8)	< 0.001	48.0 (41.5–55.1)	44.6 (38.4–52.8)	0.012
apoA-IV, μmol/L	2.2 (1.7–2.8)	2.3 (1.8–2.9)	2.2 (1.6–2.8)	0.004	2.2 (1.7–2.8)	2.3 (1.7–2.9)	0.710	2.3 (1.8–2.9)	2.1 (1.5–2.7)	0.004
apoB-100, μmol/L	1.8 (1.5–2.2)	1.7 (1.4–2.1)	1.9 (1.5–2.3)	< 0.001	1.7 (1.4–2.1)	1.9 (1.5–2.3)	< 0.001	1.8 (1.4–2.2)	1.9 (1.5–2.2)	0.265
apoC-I, μmol/L	8.0 (6.7–9.5)	8.1 (6.8–9.9)	7.8 (6.4–9.2)	0.006	7.9 (6.7–9.8)	8.1 (6.5–9.5)	0.824	8.0 (6.7–9.7)	7.7 (6.2–8.9)	0.022
apoC-III, μmol/L	16.9 (13.8–20.7)	16.9 (13.8–21.2)	16.9 (13.8–20.5)	0.827	16.3 (13.4–19.9)	17.5 (14.3–21.1)	0.001	16.9 (13.8–20.8)	16.4 (13.4–20.5)	0.373
apoE, μmol/L	1.2 (1.0–1.6)	1.2 (1.0–1.7)	1.2 (1.0–1.6)	0.711	1.2 (1.0–1.6)	1.3 (1.0–1.7)	0.076	1.2 (1.0–1.6)	1.3 (1.0–1.6)	0.687
apoJ, μmol/L	4.7 (4.1–5.3)	4.7 (4.1–5.2)	4.6 (4.0–5.4)	0.818	4.6 (4.1–5.2)	4.7 (4.1–5.4)	0.220	4.7 (4.1–5.4)	4.6 (4.0–5.2)	0.655

Values are presented as median (interquartile range) unless otherwise stated. *p* values of continuous and dichotomous variables were calculated using Mann-Whitney *U* test and χ^2 test, respectively.
Apo, apolipoprotein.

of apos A-I, A-II, A-IV, C-I, C-III, E, and TG increased postprandially by 3% (apoA-II) to 20% (apoA-IV). Highest concentration increases of 22% were seen for TG. Statin therapy significantly reduced concentrations of LDL-C (−22%), non-HDL-C (−19%), and total cholesterol (−15%). Additionally, plasma levels of apoB-100 (−16%), apoC-I (−9%), apoE (−13%), and apoA-II (−4%) decreased upon statin intake.

3.4. Plasma Apo levels in ASCVD and controls

Distributions of plasma apo concentrations in the LIFE-Heart Study are summarized in Table 3. Highest plasma levels were determined for apoA-I and apoA-II with median concentrations of 57.8 μmol/L and 47.3 μmol/L, respectively. In contrast, the overall median plasma level of lowest concentrated apoE was 1.2 μmol/L. In CAD, CAP and PAD cases plasma concentrations of apos A-I and A-II were significantly and consistently lower than in control subjects. Additionally, patients suffering from CAD or PAD showed lower plasma levels of apos A-IV and C-I than respective controls. By contrast, apoB-100 levels were elevated in CAD and CAP cases while apoC-III plasma concentrations were only increased in CAP patients.

For an analysis of associations between apos and ASCVD, we adjusted each apo for its individual influencing factors identified before. We found that apos correlated differently with the investigated manifestations of atherosclerosis as shown in Fig. 2. Significant associations with CAD were seen for apoA-I (odds ratio per one SD [OR], 0.77; 95% confidence interval [CI] 0.67–0.89), apoA-IV (OR, 0.85; 95% CI, 0.74–0.97), and apoB-100 (OR, 1.68; 95% CI, 1.44–1.97). Likewise, apos A-I (OR, 0.84; 95% CI, 0.73–0.96), A-II (OR, 0.85; 95% CI, 0.74–0.98), B-100 (OR, 1.55; 95% CI, 1.33–1.80), C-III (OR, 1.25; 95% CI, 1.09–1.44), and E (OR, 1.25; 95% CI, 1.09–1.44) indicated CAP. A negative correlation with PAD was found for apoA-IV (OR, 0.68; 95% CI, 0.54–0.86) whereas apos C-I and J showed no association with ASCVD.

Based on this analysis, we investigated whether the observed significant associations between apos and ASCVD manifestations are independent of other related lipid species (Fig. 3). First, we investigated associations with CAD. Data analysis confirmed an independent positive correlation between elevated apoB-100 concentrations and CAD risk (OR, 1.39; 95% CI, 1.05–1.84) even after adjustment for LDL-C. By contrast, the association of apoA-I with CAD did not persist after additional adjustment for HDL-C. As apoA-IV concentrations are increased postprandially, we tested the association between apoA-IV with CAD in the subset of fasted individuals. Here, we found that the association of apoA-IV with CAD (OR, 0.72; 95% CI, 0.53–0.98) was independent of HDL-C and TG (Supplemental Fig. 1). Regarding CAP, we could show that high concentrations of apos B-100 (OR, 1.55; 95% CI, 1.18–2.04), C-III (OR, 1.30; 95% CI, 1.06–1.58), and E (OR, 1.34; 95% CI, 1.13–1.58) were correlated with a higher CAP risk independently of associated lipid species. In contrast, associations of apos A-I and A-II with CAP were not independent of HDL-C. Eventually, our analysis of PAD showed that the association of apoA-IV with PAD (OR, 0.74; 95% CI, 0.58–0.95) was independent of HDL-C and TG.

4. Discussion

While the impact of apoA-I and apoB on the pathogenesis and progression of ASCVD was extensively studied over the last decades, investigations on the role of other apos like apoC-III and apoA-IV in ASCVD were up to now restricted to functional studies and small cohorts. Lately, the application of novel targeted proteomics approaches enables new insights on associations between apos and ASCVD in large clinical and epidemiological studies. However, as of now only Pechlaner et al. used a multiplexed LC-MS/MS approach to investigate the role of apos in incident ASCVD in a prospective population-based study [16]. By contrast, in the present study we investigated associations between apos and stable manifestations of ASCVD in a case-

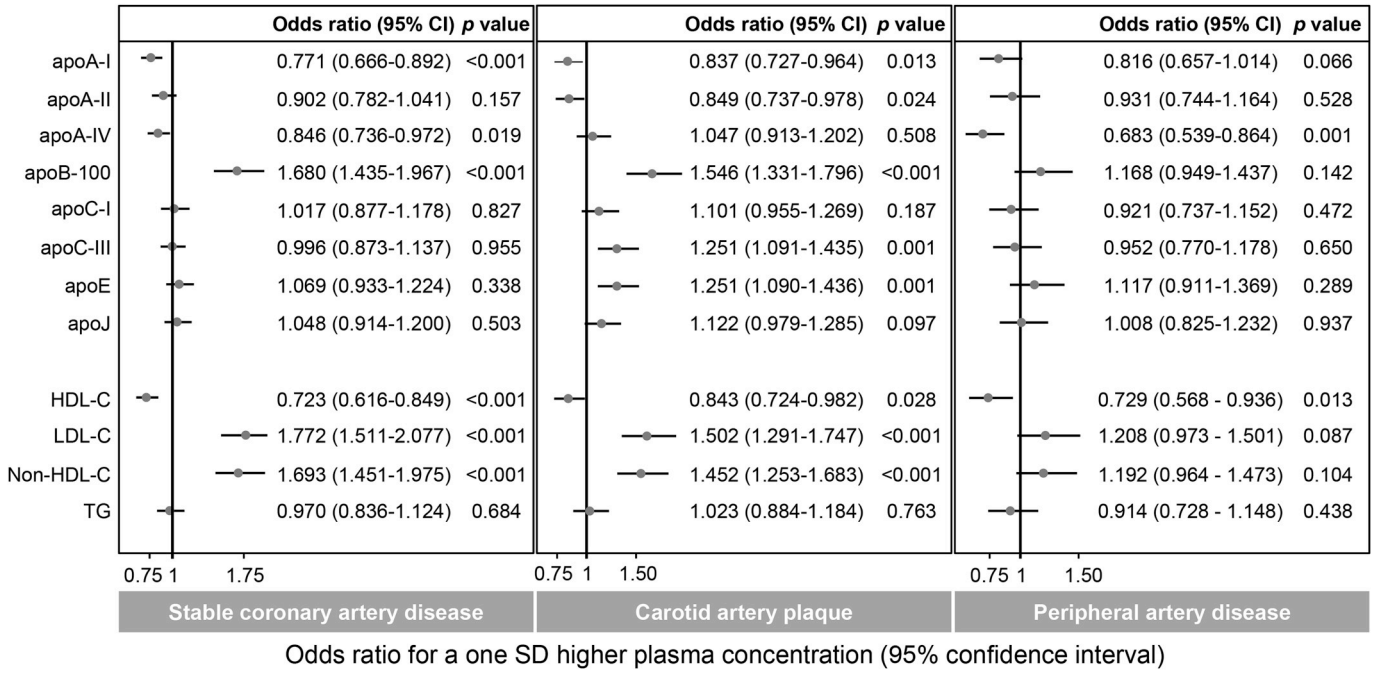

Fig. 2. Associations of apolipoproteins and lipids with stable CAD, CAP, and PAD upon adjustment for identified confounding variables.
Quantitatively, one SD corresponds to: apoA-I, 12.7 μmol/L; apoA-II, 10.9 μmol/L; apoA-IV, 0.9 μmol/L; apoB-100, 0.6 μmol/L; apoC-I, 2.2 μmol/L; apoC-III, 6.0 μmol/L; apoE, 0.5 μmol/L; apoJ, 1.0 μmol/L; HDL-C, 0.38 mmol/L; LDL-C, 1.05 mmol/L; non-HDL-C, 1.15 mmol/L; TG, 1.16 mmol/L. CI, confidence interval; other abbreviations as in Fig. 1.

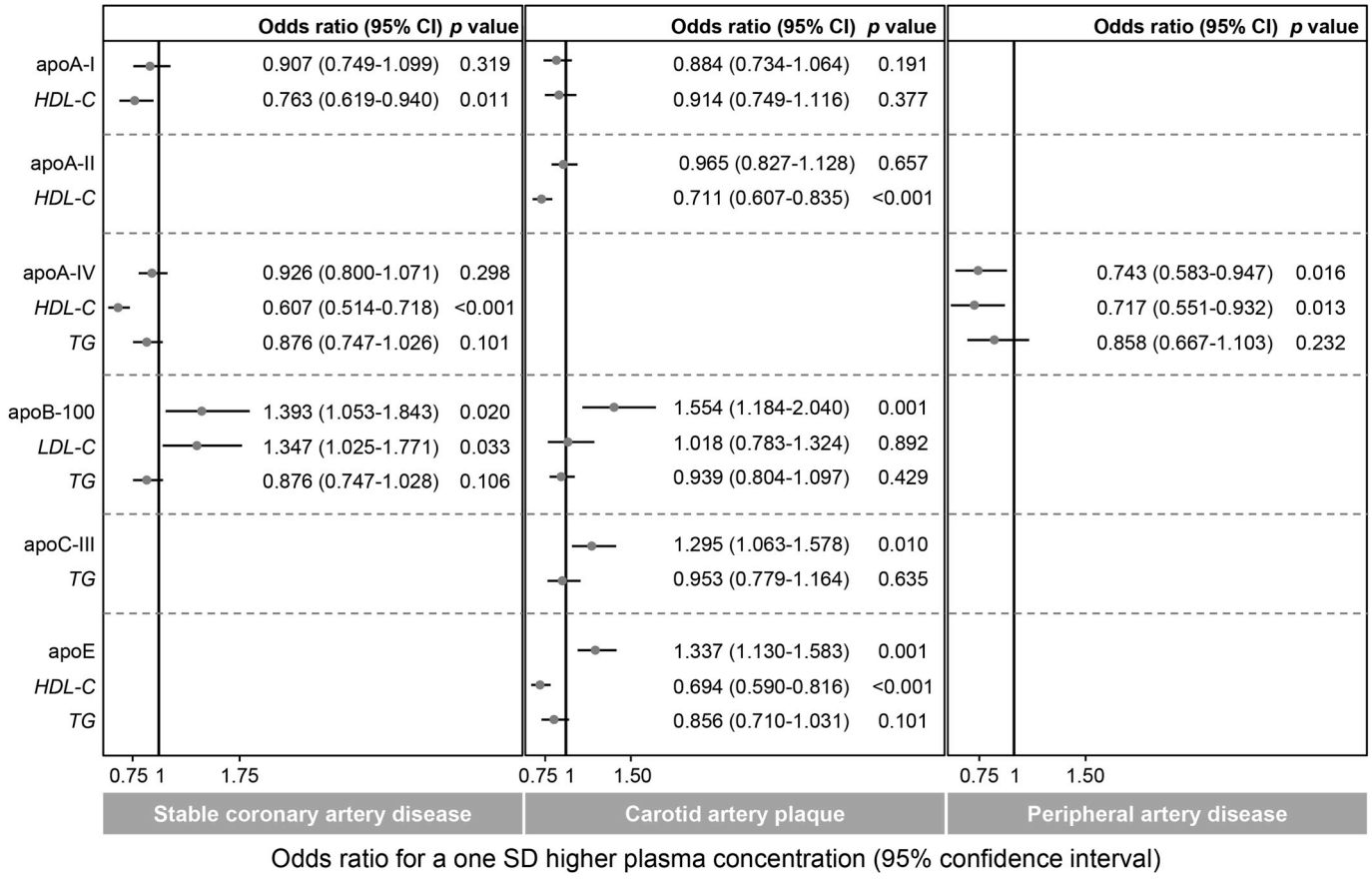

Fig. 3. Associations of apolipoproteins significantly associated with stable CAD, CAP or PAD upon additional adjustment for related lipid species.
Abbreviations and SD like in Figs. 1 and 2.

control study design. In two of the three investigated atherosclerotic manifestations apoA-IV was shown to be a protective factor. Furthermore, the role of the established risk marker apoB-100 in ASCVD was confirmed for CAD and CAP. Additionally, apos C-III and E were found to be associated with an increased risk for CAP.

The glycoprotein apoA-IV has been ascribed atheroprotective functions based on its antioxidant and anti-inflammatory properties as well as its role in several steps of the reverse cholesterol transport [22]. In our case-control study we provide valuable evidence of a relationship between low apoA-IV concentrations and the ASCVD manifestations CAD and PAD. Similar findings had previously been demonstrated solely for CAD in small groups of patients [8,10].

Physiologically, the majority of circulating apoA-IV is lipid-free or associated with chylomicrons while only minor proportions are related to HDL [10]. By the exclusion of patients with impaired kidney function, potential confounding effects on apoA-IV plasma concentrations caused by chronic kidney disease were avoided [9]. We could show that associations between apoA-IV and ASCVD are independent of HDL-C and TG in fasting individuals. One explanation of the inverse association of apoA-IV with ASCVD is its involvement in reverse cholesterol transport. ApoA-IV was shown to promote the cholesterol efflux from cholesterol-loaded monocytes in *APOA4* transgenic mice [23], which is in line with findings on stabilized fibrous caps and smaller lipid cores of atherosclerotic plaques in *Apoe*-deficient mice infused with human lipid-free apoA-IV [24]. It was further previously demonstrated that the apoA-IV-inherent potential to inhibit LDL oxidation is modified by *APOA4* polymorphisms [25]. Additionally, mutations of human *APOA4* resulting in low apoA-IV plasma concentrations were shown to be associated with a significantly increased risk for CAD [26].

In accordance with the findings of Pechlaner et al. [16] we also demonstrated apoC-III and apoE as vascular risk factors, which were exclusively associated with CAP. Recently, apoC-III was identified as potential therapeutic target in hypertriglyceridemia [16,27,28] since apoC-III-related inhibition of LPL impairs lipolysis of chylomicrons and VLDL [28]. However, a correlation between apoC-III and coronary atherosclerosis could not be observed in our study. This discrepancy to earlier investigations [3,25] may be due to different clinical phenotypes, e.g. stable CAD versus acute MI.

Besides apoC-III, apoE is another key regulator of the clearance of apoB-containing lipoproteins [29]. ApoE is a protein with three genetically determined polymorphisms. The functionalities of the resulting apoE isoforms apoE2 and apoE4 are altered in comparison to wild-type apoE3 and both isoforms are well known risk markers of CAD [30]. Generally, apoE is believed to be anti-atherogenic due to its central role in lipoprotein metabolism and transport [31]. Contrary to this assumption, we found a positive association between plasma levels of apoE and CAP, which is, however, in accordance with previous results of the PREVEND study that identified apoE as risk marker of incident CAD in women [32].

Differential post-translational modifications of the glycoproteins apoA-IV [33] and apoC-III [34] may influence their role in ASCVD. However, a distinction of different proteoforms could not be performed in the presented study.

In summary, we investigated three different manifestations of atherosclerosis to clarify the question if apos are independent risk factors of stable manifestations of atherosclerosis. Therefore, a comprehensive analysis of confounding clinical covariates was performed to allow a reliable risk evaluation. Apos were differentially associated with the considered atherosclerotic manifestations. While apoA-IV was shown to be atheroprotective, apos B-100, C-III, and E were associated with higher risk for atherosclerosis, independently of standard lipid parameters. Mass spectrometric profiling of apolipoprotein concentrations might serve as a valuable tool in the risk assessment of distinct atherosclerotic diseases.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

JD performed apo measurements, analyzed the data and wrote the manuscript; FB and AT conducted the study and critically reviewed the manuscript; JT and GS designed the study and critically reviewed the manuscript, MS supervised the data analysis and contributed to manuscript writing, RB contributed to the data acquisition and critically reviewed the manuscript. UC designed the study, supervised the data analysis and drafted the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.11.006.

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