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ARTICLE

Plasma concentrations of lipoproteins and risk of lower-limb peripheral artery disease in people with type 2 diabetes: the SURDIAGENE study

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Abstract

Aims/hypothesis The lipid profile has not been fully investigated in individuals with peripheral artery disease (PAD). We aimed to evaluate the relationship between plasma concentrations of lipoproteins and the prevalence of lower-limb PAD at baseline and its incidence during follow-up in people with type 2 diabetes.

Methods Plasma concentrations of total cholesterol, HDL-cholesterol, triacylglycerol and apolipoprotein (Apo) A-I, ApoA-II, ApoB-100 and Apo(a) were measured at baseline using colorimetric or MS methods in the SURDIAGENE cohort. Total cholesterol/HDL-cholesterol ratio, non-HDL-cholesterol and LDL-cholesterol were estimated using computation formulas. Logistic and Cox proportional hazard regression models were fitted to estimate OR or HR, with related 95% CI, for baseline prevalence or incidence of major PAD (lower-limb amputation or requirement of revascularisation) during follow-up by increasing lipoprotein tertiles, after adjustment for key confounders.

Results Among 1468 participants (women 42%, mean ± SD age 65 ± 11 years, duration of diabetes 14 ± 10 years at baseline), 129 (8.8%) had a baseline history of major PAD. Major PAD was less prevalent at baseline in the highest (vs lowest) tertile of HDL-cholesterol (OR 0.42 [95% CI 0.26, 0.71], $p = 0.001$) and ApoA-I (OR 0.39 [95% CI 0.23, 0.67], $p = 0.0007$), and more frequent in the highest tertile of total cholesterol/HDL-cholesterol ratio (OR 1.95 [95% CI 1.18, 3.24], $p = 0.01$). Among 1339

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Research in context

What is already known about this subject?

- Dyslipidaemia affects 50% of individuals with type 2 diabetes and has been linked to both coronary and cerebrovascular atherosclerotic diseases
- Few studies have examined the relationship between dyslipidaemia and peripheral artery disease (PAD) in people with type 2 diabetes, with contrasting results

What is the key question?

- What is the profile of lipoproteins associated with major PAD in individuals with type 2 diabetes?

What are the new findings?

- Major PAD (lower-limb amputation or revascularisation) was less prevalent at baseline in the top (vs bottom) tertile of HDL-cholesterol and ApoA-I, and more frequent in the top tertile of total cholesterol/HDL-cholesterol ratio
- Among 1339 participants without a baseline history of PAD, the incidence of major PAD decreased in the top (vs bottom) tertile of HDL-cholesterol or ApoA-I and increased in the top tertile of total cholesterol/HDL-cholesterol ratio and non-HDL-cholesterol
- These associations were independent of putative confounders and mainly reliable after treating all-cause death or coronary events as competing risks

How might this impact on clinical practice in the foreseeable future?

- Individuals with type 2 diabetes and low plasma concentrations of HDL-cholesterol and ApoA-I or high total cholesterol/HDL-cholesterol ratio and non-HDL-cholesterol should be more strongly monitored to prevent the development of major adverse limb events

participants without a history of PAD at baseline, incident PAD occurred in 97 (7.2%) during a median (25th–75th percentile) duration of follow-up of 7.1 (4.4–10.7) years, corresponding to 9685 person-years and an incidence rate of 9.8 (95% CI 8.0, 12.0) per 1000 person-years. The risk of incident PAD was lower in the top (vs bottom) tertile of HDL-cholesterol (HR 0.54 [95% CI 0.30, 0.95], $p = 0.03$) or ApoA-I (HR 0.50 [95% CI 0.28, 0.86], $p = 0.01$) and higher in the top tertile of total cholesterol/HDL-cholesterol ratio (HR 2.81 [95% CI 1.61, 5.04], $p = 0.0002$) and non-HDL-cholesterol (HR 1.80 [95% CI 1.06, 3.12], $p = 0.03$).
Conclusions/interpretation We reported independent associations between HDL-cholesterol, ApoA-I, total cholesterol/HDL-cholesterol ratio or non-HDL-cholesterol and the prevalence or the incidence of major PAD in people with type 2 diabetes. Our findings provide a picture of lipoprotein profile in people with type 2 diabetes.

Keywords Apolipoproteins · Limb loss · Lipids · Lipoproteins · Lower-limb amputation · Peripheral arterial disease · Revascularisation · Type 2 diabetes

Abbreviations

ACR	Albumin/creatinine ratio
Apo(a)	Apolipoprotein (a)
ApoA-I	Apolipoprotein A-I
ApoA-II	Apolipoprotein A-II
ApoB-100	Apolipoprotein B100
Lp(a)	Lipoprotein (a)
PAD	Peripheral artery disease
SURDIAGENE	SURVIe, DIAbete de type 2 et GENEtique

Introduction

Lower-limb peripheral artery disease (PAD) is an emerging public health burden with an endemic progression worldwide resulting from demographic expansion, population ageing and growing prevalence of type 2 diabetes and smoking habits [1, 2]. PAD is more prevalent in individuals with type 2 diabetes than in people without diabetes, with a poor prognosis leading to negative impacts on individual quality of life, healthcare

systems and societies [3–5]. PAD is responsible for a dramatic increase in risk of non-traumatic lower-limb amputation, 5–12 times higher in individuals with diabetes than in those without a history of diabetes [3, 6]. PAD is also associated with excess risk of CVD and non-CVD with a significant reduction in life expectancy [5, 7, 8].

Dyslipidaemia affects about 50% of individuals with type 2 diabetes and is a major independent and modifiable risk factor for ischaemic CVD [9]. Atherogenic dyslipidaemia has been linked to both coronary and cerebrovascular atherosclerotic localisations [10, 11]. Despite considerable research on lipid metabolism and its impact in the development of CVD, only few reports dealt with PAD in individuals with diabetes. The specific lipoprotein components that contribute to PAD are not clearly established. In the present study, we investigated the profile of lipoproteins associated with the prevalence of major PAD at baseline and its incidence during follow-up in individuals with type 2 diabetes. Hence, we measured plasma concentrations of a range of lipid variables, including total cholesterol, HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol, triacylglycerol, apolipoprotein A-I (ApoA-I), apolipoproten A-II (ApoA-II), apolipoprotein B100 (ApoB-100) and lipoprotein (a) [Lp(a)] at baseline, and we investigated their relationships with the prevalence and the incidence of major PAD in a prospective cohort of individuals with type 2 diabetes.

Methods

Participants SURDIAGENE (SURvie, DIAbete de type 2 et GENEtique) is a French single-centre prospective cohort designed to investigate genetic and biochemical determinants of vascular complications among 1468 inpatient participants with type 2 diabetes diagnosed for at least 2 years [12]. The main exclusion criteria were the existence of a non-diabetic kidney disease and short follow-up duration (<1 year). Participants were recruited at the University Hospital of Poitiers, France, from 2002 to 2012, and were prospectively followed-up until death, or until 31 December 2015. The study protocol was approved by the Poitiers University Hospital Ethics Committee (CPP Ouest 3) and all participants gave written informed consent. The associations between lipoproteins and a baseline history of major PAD were tested in the whole cohort. Then, we tested the associations between lipoproteins and the incidence of major PAD in the incidence cohort, after exclusion of 129 individuals with a baseline history of PAD. The associations between lipoproteins and secondary endpoints (see below) were tested in participants without a baseline history of lower-limb amputation or revascularisation procedure as appropriate (electronic supplementary material [ESM] Fig. 1).

Assessments of plasma concentrations of lipoproteins Plasma concentrations of total cholesterol, HDL-cholesterol and triacylglycerol were determined centrally at baseline in the fasting state using a colorimetric method, running on an automated analyser (Kone Optima; Thermo Clinical Labsystems, Vantaa, Finland). The total cholesterol/HDL-cholesterol ratio was calculated. Plasma concentrations of LDL-cholesterol were estimated using the Friedewald formula in 1409 participants with triacylglycerol <4.5 mmol/l. Non-HDL-cholesterol was calculated as total cholesterol value minus HDL-cholesterol. ApoA-I, ApoA-II, ApoB-100 and apolipoprotein (a) [Apo(a)] were quantified in plasma samples (40 μl) using a validated multiplexed assay involving trypsin proteolysis and the subsequent analysis of proteotypic peptides by LC-MS/MS [13, 14]. The intra- and inter-assay imprecisions of the analytical method were assessed throughout experiments and were below 6.4% for all targeted apolipoproteins. Since Lp(a) consists of a single apolipoprotein [Apo(a)] bound to the ApoB-100 moiety of an LDL-like particle, the molar concentrations of Apo(a) were assumed to be equivalent to those of Lp(a). Lp(a) concentrations in units of nmol/l were then converted to units of mg/l using the following formula: Lp(a) (nmol/l) = 0.218 × Lp(a) (mg/l) − 3.83 [15]. Hence, results are presented in this work as Lp(a) expressed in mg/l.

Definition of clinical conditions at baseline The history of tobacco smoking was defined as never, former or current smokers. Diabetic retinopathy was staged as absent, non-proliferative or proliferative. The history of macrovascular disease was defined as the presence of at least one of the following conditions: myocardial infarction; stable angina; stroke; transient ischaemic attack; or coronary or carotid arterial revascularisation. The prevalence of lower-limb PAD was defined as the history of minor (at least one toe or transmetatarsal) or major (transtibial or transfemoral) amputation, or revascularisation at baseline.

Definition of endpoints during follow-up The primary endpoint, incident major PAD, was defined as the first occurrence either of lower-limb amputation (transmetatarsal, transtibial or transfemoral) or the requirement of a lower-limb revascularisation procedure (angioplasty or surgery) during follow-up. Revascularisation and lower-limb amputation were considered individually as secondary endpoints.

Adjudication procedure Outcomes were determined from participants' medical records and interviews with their general practitioners every second year from 2007. The hospitalisation records and all other relevant supporting documents were used to adjudicate clinical outcomes. Each endpoint was centrally reviewed by an independent adjudication committee.

Causes of lower-limb amputation We have examined participants' medical files to determine the potential causes of lower-limb amputation at baseline (for prevalent amputation) and at the endpoint time (for incident amputation): neuropathy (as reported by the investigator); PAD (abolition of peripheral pulses, intermittent claudication, lower-limb artery stenosis >50% with haemodynamic effects in ultrasound examination); and/or foot infection (skin, soft tissue, bone or joint).

Statistical analyses Categorical variables are expressed as the number of participants with corresponding percentage. Continuous variables are expressed as mean ± SD or median (25th–75th percentile) for those with skewed distribution. Comparisons of characteristics of participants at baseline were performed using χ^2, ANOVA or Wilcoxon tests. The correlation between different lipoproteins were assessed using Pearson or Spearman's rank test. Plasma concentrations of lipoproteins were categorised into three equal increasing tertiles: first (T1, lowest tertile); second (T2, middle tertile); and third (T3, highest tertile). Missing data were rare (Table 1) and were removed from all analyses that included the covariate.

Logistic regression models were used to test the associations between lipoproteins and the prevalence of major PAD at baseline, expressed as OR with related 95% CI for T2 vs T1 and T3 vs T1. Analyses were adjusted for every potential confounding variable that was nominally associated ($p < 0.10$) with the prevalence of major PAD at baseline in the univariate comparisons: sex; age; duration of diabetes; BMI; systolic and diastolic BP; HbA_{1c}; urinary albumin/creatinine ratio (ACR, using a natural log transformation); eGFR (estimated using the Chronic Kidney Disease–Epidemiology Collaboration equation); history of tobacco smoking (never, former, current); history of diabetic retinopathy; history of macrovascular disease; and history of medication use (antihypertensive, antiplatelet or anticoagulant drugs, statins, fibrates or metformin).

Restricted cubic splines (10th, 25th, 75th and 90th percentiles as knots and the median as reference) analyses were plotted to check for the linearity in the relationship between plasma concentrations of lipoproteins at baseline and the risk of incident PAD during follow-up.

Kaplan–Meier curves were used to plot the incidence of endpoints according to tertiles of lipoproteins at baseline and compared using logrank test. Cox proportional hazards regression models were computed to calculate HRs, with related 95% CIs, for endpoints during follow-up by tertiles of lipoproteins at baseline (T2 vs T1 and T3 vs T1). Lipid variables with a linear PAD relationship were also tested as continuous variables (HR for the primary endpoint by each single SD increase). Analyses were adjusted for age plus every potential confounding variable that was nominally associated ($p < 0.10$) with the incidence of major PAD during follow-up in the univariate comparisons: sex; duration of diabetes; BMI; systolic BP; ACR; eGFR; history of tobacco smoking (never, former, current); diabetic retinopathy; and use of antihypertensive, statin, metformin and insulin therapies. We tested interaction between relevant lipid variables in their association with the primary endpoint by including them and their product within the Cox model. We also tested interaction between lipoproteins and the use of statins in PAD association. The proportional hazards assumption was checked using the Schoenfeld residuals method (all p values >0.05).

As sensitivity analyses, we estimated the risk of the primary endpoint by plasma concentrations of lipoproteins after treating all-cause death or coronary events (myocardial infarction or requirement of coronary revascularisation, whichever came first) as competing risk using the Fine and Gray method.

Statistical analysis was performed using JMP software, version 14.0 (SAS Institute, Cary, NC, USA; www.sas.com) and Stata software version 15.1 (StataCorp, TX, USA; http://www.stata.com).

Results

Characteristics of participants at baseline Among 1468 patients enrolled in SURDIAGENE, 42% were women and 10% were current smokers at baseline. The mean ± SD age and duration of diabetes were 65 ± 11 years and 14 ± 10 years, respectively. The mean ± SD plasma concentrations of lipids and lipoproteins were as follows: total cholesterol 4.8 ± 1.2 mmol/l; HDL-cholesterol 1.2 ± 0.4 mmol/l; non-HDL-cholesterol 3.6 ± 1.2 mmol/l; LDL-cholesterol 2.7 ± 1.0 mmol/l; ApoA-I 1.3 ± 0.3 g/l; ApoA-II 0.31 ± 0.11 g/l; and ApoB-100 0.81 ± 0.29 g/l. The median (25th–75th percentiles) of total cholesterol/HDL-cholesterol ratio, triacylglycerol and Lp(a) were 4.0 (3.1–5.2), 1.5 (1.1–2.3) mmol/l and 74 (19–203) mg/l, respectively. Plasma concentrations of lipoproteins in different corresponding tertiles are presented in ESM Table 1, and the pairwise correlations are displayed in ESM Table 2.

Prevalence of major PAD by plasma concentrations of lipoproteins at baseline A history of major PAD was reported at baseline in 129 (8.8%) patients. Table 1 shows the characteristics of participants by the baseline prevalence of major PAD. The mean ± SD plasma concentrations of HDL-cholesterol (1.1 ± 0.3 vs 1.2 ± 0.4 mmol/l, $p = 0.003$), ApoA-I (1.2 ± 0.2 vs 1.3 ± 0.3 g/l, $p < 0.0001$) and ApoA-II (0.28 ± 0.10 vs 0.32 ± 0.11 g/l, $p = 0.0002$) were significantly lower in participants who had a history of major PAD at baseline compared with those who did not (Table 1). PAD at baseline was less prevalent in the highest compared with the lowest tertile of HDL-cholesterol, Apo-A1 and Apo-A2, and more frequent in the highest tertile of total cholesterol/HDL-cholesterol ratio

Table 1 Characteristics of participants at baseline according to the prevalence and the incidence of major PAD

Characteristic			Prevalence of major PAD at baseline			Incidence of major PAD during follow-up[a]		
	Overall	Missing data (*n*)	No	Yes	*p* value	No	Yes	*p* value
N	1468		1339	129		1242	97	
Clinical variables								
Women	620 (42)	0	591 (44)	29 (22)	<0.0001	570 (46)	21 (22)	<0.0001
Age, years	65 ± 11	0	64 ± 11	68 ± 9	<0.0001	64 ± 11	66 ± 10	0.26
Duration of diabetes, years	14 ± 10	2	14 ± 10	18 ± 11	<0.0001	14 ± 10	17 ± 10	0.01
BMI, kg/m^2	31 ± 6	0	31 ± 6	29 ± 6	<0.0001	32 ± 6	30 ± 5	0.009
Heart rate, beats/min	71 ± 14	8	71 ± 14	72 ± 15	0.27	71 ± 14	70 ± 14	0.56
SBP, mmHg	132 ± 18	7	132 ± 17	136 ± 20	0.01	132 ± 17	137 ± 18	0.005
DBP, mmHg	72 ± 11	7	73 ± 11	71 ± 11	0.08	73 ± 11	72 ± 12	0.39
Biological variables								
HbA_{1c}, %	7.8 ± 1.5	1	7.8 ± 1.6	7.5 ± 1.3	0.02	7.8 ± 1.6	7.9 ± 1.6	0.53
HbA_{1c}, mmol/mol	62 ± 17		62 ± 17	58 ± 14		62 ± 17	63 ± 17	
Urinary ACR (mg/mmol)	3 (1–14)	18	3 (1–12)	9 (2, 74)	<0.0001	3 (1–11)	9 (2–69)	<0.0001
eGFR, ml min^{-1} [1.73 m]$^{-2}$	73 ± 25	0	74 ± 25	63 ± 26	<0.0001	74 ± 24	64 ± 30	0.0002
Total cholesterol, mmol/l	4.8 ± 1.2	0	4.8 ± 1.2	4.6 ± 1.1	0.07	4.8 ± 1.2	4.9 ± 1.3	0.20
HDL-cholesterol, mmol/l	1.2 ± 0.4	8	1.2 ± 0.4	1.1 ± 0.3	0.003	1.2 ± 0.4	1.1 ± 0.4	0.03
Total cholesterol/HDL-cholesterol ratio	4.0 (3.1–5.2)	8	4.0 (3.1–5.1)	4.3 (3.3–5.4)	0.12	4.0 (3.1–5.1)	4.4 (3.5–5.8)	0.006
Non-HDL-cholesterol, mmol/l	3.6 ± 1.2	8	3.6 ± 1.2	3.5 ± 1.1	0.44	3.6 ± 1.2	3.9 ± 1.3	0.02
LDL-cholesterol, mmol/l	2.7 ± 1.0	59[b]	2.7 ± 1.0	2.7 ± 0.9	0.57	2.7 ± 1.0	2.9 ± 1.0	0.03
Triacylglycerol, mmol/l	1.5 (1.1–2.3)	5	1.5 (1.1–2.3)	1.5 (1.1–2.2)	0.98	1.5 (1.1–2.3)	1.7 (1.1–2.3)	0.48
ApoA-I, g/l	1.3 ± 0.3	2	1.3 ± 0.3	1.2 ± 0.2	<0.0001	1.3 ± 0.3	1.2 ± 0.3	0.02
ApoA-II, g/l	0.31 ± 0.11	2	0.32 ± 0.11	0.28 ± 0.10	0.0002	0.32 ± 0.11	0.30 ± 0.13	0.14
ApoB-100, g/l	0.81 ± 0.29	2	0.81 ± 0.29	0.80 ± 0.32	0.66	0.81 ± 0.29	0.82 ± 0.31	0.84
Lp(a), mg/l	74 (19–203)	2	73 (19–200)	94 (20–297)	0.06	72 (19–193)	104 (24–290)	0.03
History of tobacco smoking								
Never	759 (52)	0	718 (54)	41 (32)	<0.0001	684 (55)	34 (35)	0.0002
Former	556 (38)	0	482 (36)	74 (57)		437 (35)	45 (46)	
Current	153 (10)	0	139 (10)	14 (11)		121 (10)	18 (19)	
No. of cigarette packs/ year	25 (10–40)	111	25 (10–40)	30 (15–45)	0.009	25 (10–40)	30 (15–42)	0.51
Medical history								
Diabetic retinopathy	639 (44)	22[c]	558 (42)	81 (63)	<0.0001	495 (40)	63 (65)	<0.0001
Macrovascular disease	527 (36)	0	456 (34)	71 (55)	<0.0001	416 (33)	40 (41)	0.15
History of medication use								
Antihypertensive drugs	1215 (83)	0	1095 (82)	120 (93)	0.0006	1006 (81)	89 (92)	0.006
Statin	666 (45)	0	598 (45)	68 (53)	0.09	544 (44)	54 (56)	0.03
Fibrate	162 (11)	0	158 (12)	4 (3)	0.001	151 (12)	7 (7)	0.19
Antiplatelet or anticoagulant drug	617 (42)	0	535 (40)	82 (64)	<0.0001	489 (39)	46 (47)	0.13
Use of metformin	690 (47)	0	654 (49)	36 (28)	<0.0001	619 (50)	35 (36)	0.01
Use of insulin therapy	883 (60)	0	797 (60)	86 (67)	0.13	728 (59)	69 (71)	0.02

Data are presented as *n* (%) or mean ± SD, or as median (25th–75th percentiles) for variables with skewed distribution (urinary albumin to creatinine ratio, triacylglycerol, Lp(a), total cholesterol/HDL-cholesterol ratio and number of cigarette packs per year)

[a] Analyses performed in participants without a history of major PAD at baseline

[b] Includes 51 participants who were excluded from the estimation (using the Friedewald formula) because plasma concentrations of triacylglycerol were high (>4.5 mmol/l)

[c] Includes missing data and undetermined retinopathy status

Comparisons of qualitative and quantitative variables were performed using χ^2 and ANOVA tests, respectively. Wilcoxon test was used for comparisons of variables with skewed distribution. $p<0.05$ was considered as significant

DBP, diastolic BP; SBP, systolic BP

(Table 2; T3 vs T1). Logistic regression models confirmed these associations after adjustment for confounding variables (Table 2).

Prevalence of lower-limb amputation and revascularisation by plasma concentrations of lipoproteins at baseline A history of lower-limb amputation (74% minor and 26% major) was reported at baseline in 73 (5.0%) participants. They all had evidence of PAD (at least one of the following: abolition of peripheral pulses 62%, intermittent claudication 42%, lower-limb artery stenosis >50% with haemodynamic effects 59%). Peripheral diabetic neuropathy and foot infection were also reported at baseline in 59% and 68% of participants with a history of amputation. A history of lower-limb revascularisation procedures was reported at baseline in 73 (5.0%) participants. The highest tertiles of HDL-cholesterol and ApoA-I, compared with the respective lowest tertiles, were significantly associated with lower prevalence of lower-limb amputation and revascularisation at baseline (ESM Table 3).

Incidence of major PAD during follow-up by plasma concentrations of lipoproteins at baseline Among 1339 participants without a history of PAD at baseline, incident PAD occurred in 97 (7.2%) during a median (25th–75th percentile) duration of follow-up of 7.1 (4.4–10.7) years, corresponding to 9685 person-years and an incidence rate of 9.8 (95% CI 8.0, 12.0) per 1000 person-years. Characteristics of participants at baseline by incident PAD during follow-up are presented in Table 1.

Plasma concentrations of HDL-cholesterol and ApoA-I were significantly lower, while LDL-cholesterol, non-HDL-cholesterol and Lp(a) were higher in participants who experienced a major PAD during follow-up compared with participants who had not (Table 1). The total cholesterol/HDL-cholesterol ratio was also higher in participants who experienced major PAD during follow-up. The relationships between plasma concentrations of each lipid biomarker and the risk of major PAD during follow-up were not log-linear, except for ApoA-I (ESM Fig. 2).

The Kaplan–Meier estimate of 10 year cumulative incidence (95% CI) of major PAD was significantly lower for participants in the highest (T3) vs lowest tertile (T1) of HDL-cholesterol (T1, 11.4 [8.0, 15.8]%; T2, 10.8 [7.6, 15.3]%; T3, 6.9 [4.4, 10.6]%) and ApoA-I (T1, 13.1 [9.0, 18.1]%; T2, 10.1 [7.0, 14.3]%; T3, 6.4 [4.1, 10.0]%) (Table 2, Fig. 1b,g). It was also reduced in the middle vs the lowest ApoA-II tertile (T1, 12.8 [9.2, 17.4]%; T2, 7.6 [4.8, 11.7]%; T3, 9.0 [6.1, 13.0]%) and increased in the highest vs lowest tertile of total cholesterol/HDL-cholesterol ratio (T1, 6.4 [4.0, 10.1]%; T2, 9.1 [6.2, 13.1]%; T3, 13.3 [9.2, 17.9]%) (Table 2, Fig. 1c,h). HDL-cholesterol, total cholesterol/HDL-cholesterol ratio and ApoA-I (but not ApoA-II) remained significantly associated with the risk of major PAD after adjusting for key confounders (Table 2). These associations remained significant after considering all-cause death or coronary events as competing risks (ESM Table 4). Each single SD increase in ApoA-I was significantly associated with a reduced risk of major PAD (HR 0.76 [95% CI 0.60, 0.96], $p = 0.02$).

The highest tertile of non-HDL-cholesterol was also significantly associated with increased risk of major PAD after adjusting for confounders. However, this association did not persist after treating all-cause death or coronary events as competing risk (ESM Table 4). No other significant association was observed between lipids and major PAD (Table 2).

We observed a significant interaction between total cholesterol/HDL-cholesterol ratio and non-HDL-cholesterol in their association with the risk of incident PAD (p for interaction = 0.01). No other significant interaction was observed between HDL-cholesterol, total cholesterol/HDL-cholesterol ratio, non-HDL-cholesterol or ApoA-I in their association with the risk of major PAD (p values >0.05). In addition, no significant interaction was observed between HDL-cholesterol (p = 0.49), total cholesterol/HDL-cholesterol ratio ($p = 0.86$), non-HDL-cholesterol ($p = 0.06$) or ApoA-I ($p = 0.16$) with the use of statins in their association with major PAD.

Risks of incident lower-limb amputation and revascularisation by plasma concentrations of lipoproteins at baseline Lower-limb amputation (45% minor and 55% major) occurred during follow-up in 55 (3.9%) participants without a baseline history of limb loss. Its incidence rate was 5.2 (95% CI 4.0, 6.8) per 1000 person-years. Every participant who experienced limb loss during follow-up showed evidence of PAD at the time of outcomes (at least one of the following: abolition of peripheral pulses 76%, intermittent claudication 56%, lower-limb artery stenosis >50% with haemodynamic effects 95%). Peripheral diabetic neuropathy and foot infection were reported in 76% and 51% participants, respectively, among amputees at the time of endpoint. Requirement of revascularisation occurred during follow-up in 78 (5.6%) participants without a history of this procedure at baseline. Its incidence rate was 7.7 (95% CI 6.2, 9.7) per 1000 person-years. The risks of incident amputation and requirement of revascularisation, considered separately as secondary endpoints, were lower in the top tertiles of HDL-cholesterol and ApoA-I, and higher in the top tertile of total cholesterol/HDL-cholesterol ratio, compared with the bottom tertiles (Table 3).

Discussion

In the present study, we investigated the relationship between lipid variables and the prevalence of PAD at baseline and its incidence during follow-up in individuals with type 2 diabetes. We observed lower prevalent and incident PAD in participants

Table 2 Prevalence and incidence of PAD by tertiles of plasma lipoprotein concentration at baseline

Variable	Prevalent major PAD at baseline[a]				Incident major PAD during follow-up[b]				
	No, *n*	Yes, *n* (%)	OR (95% CI)	*p* value	No, *n*	Yes, *n* (%)	10 year cumulative incidence, % (95% CI)	HR (95% CI)	*p* value
Total cholesterol									
First tertile	432	57 (11.7)	Reference		415	31 (7.0)	10.2 (6.9, 14.9)	Reference	
Second tertile	449	40 (8.2)	0.93 (0.58, 1.49)	0.76	420	26 (5.8)	7.0 (4.5, 10.7)	1.01 (0.58, 1.73)	0.98
Third tertile	458	32 (6.5)	0.68 (0.40, 1.14)	0.14	407	40 (9.0)	11.8 (8.5, 16.0)	1.34 (0.81, 2.25)	0.25
HDL-cholesterol									
First tertile	428	58 (11.9)	Reference		406	37 (8.4)	11.4 (8.0, 15.8)	Reference	
Second tertile	447	40 (8.2)	0.63 (0.40, 1.00)	0.05	408	36 (8.1)	10.8 (7.6, 15.3)	1.00 (0.62, 1.62)	0.98
Third tertile	456	31 (6.4)	0.42 (0.26, 0.71)	0.001	422	22 (4.9)	6.9 (4.4, 10.6)	0.54 (0.30, 0.95)	0.03
Total cholesterol/HDL-cholesterol ratio									
First tertile	448	38 (7.8)	Reference		423	20 (4.5)	6.4 (4.0, 10.1)	Reference	
Second tertile	447	40 (8.2)	1.32 (0.80, 2.18)	0.28	411	33 (7.4)	9.1 (6.2, 13.1)	1.83 (1.04, 3.29)	0.04
Third tertile	436	51 (10.5)	1.95 (1.18, 3.24)	0.01	402	42 (9.5)	13.3 (9.2, 17.9)	2.81 (1.61, 5.04)	0.0002
Non-HDL-cholesterol									
First tertile	436	50 (10.3)	Reference		419	25 (5.6)	8.4 (5.4, 12.8)	Reference	
Second tertile	446	41 (8.4)	0.99 (0.61, 1.58)	0.95	414	30 (6.8)	8.0 (5.3, 11.7)	1.30 (0.75, 2.26)	0.35
Third tertile	449	38 (7.8)	1.04 (0.63, 1.74)	0.86	403	40 (9.0)	12.2 (8.8, 16.5)	1.80 (1.06, 3.12)	0.03
LDL-cholesterol									
First tertile	418	51 (10.9)	Reference		404	24 (5.6)	7.8 (5.1, 11.8)	Reference	
Second tertile	437	33 (7.0)	0.81 (0.49, 1.34)	0.40	398	31 (7.2)	9.0 (6.0, 13.1)	1.41 (0.81, 2.48)	0.22
Third tertile	431	39 (8.3)	0.97 (0.58, 1.61)	0.89	392	37 (8.6)	11.2 (8.0, 15.5)	1.71 (0.99, 3.00)	0.05
Triacylglycerol									
First tertile	448	41 (8.4)	Reference		415	29 (6.5)	7.6 (5.0, 11.3)	Reference	
Second tertile	444	44 (9.0)	1.23 (0.76, 1.99)	0.39	413	32 (7.2)	11.0 (7.0, 15.7)	1.22 (0.73, 2.04)	0.45
Third tertile	444	44 (9.0)	1.57 (0.95, 2.59)	0.07	411	34 (7.6)	10.1 (7.1, 14.1)	1.35 (0.81, 2.27)	0.25
ApoA-I									
First tertile	431	57 (11.7)	Reference		404	41 (9.2)	13.1 (9.0, 18.1)	Reference	
Second tertile	441	48 (9.8)	0.90 (0.58, 1.40)	0.64	412	34 (7.6)	10.1 (7.0, 14.3)	0.82 (0.51, 1.31)	0.42
Third tertile	465	24 (4.9)	0.39 (0.23, 0.67)	0.0007	425	21 (4.7)	6.4 (4.1, 10.0)	0.50 (0.28, 0.86)	0.01
ApoA-II									
First tertile	426	62 (12.7)	Reference		403	42 (9.4)	12.8 (9.2, 17.4)	Reference	
Second tertile	451	38 (7.8)	0.71 (0.45, 1.13)	0.15	422	24 (5.4)	7.6 (4.8, 11.7)	0.61 (0.36, 1.02)	0.06
Third tertile	460	29 (5.9)	0.59 (0.36, 0.98)	0.04	416	30 (6.7)	9.0 (6.1, 13.0)	0.90 (0.55, 1.48)	0.69
ApoB-100									
First tertile	440	48 (9.8)	Reference		409	36 (8.1)	11.1 (7.8, 15.5)	Reference	
Second tertile	450	39 (8.0)	0.99 (0.61, 1.59)	0.97	419	27 (6.1)	8.4 (5.5, 12.6)	0.87 (0.52, 1.44)	0.58
Third tertile	447	42 (8.6)	1.35 (0.84, 2.19)	0.22	413	33 (7.4)	9.7 (6.8, 13.8)	1.21 (0.74, 1.98)	0.43
Lp(a)									
First tertile	451	37 (7.6)	Reference		419	27 (6.1)	8.7 (5.7, 12.9)	Reference	
Second tertile	450	39 (8.0)	1.05 (0.63, 1.73)	0.85	418	28 (6.3)	9.0 (6.0, 13.1)	0.79 (0.46, 1.37)	0.40
Third tertile	436	53 (10.8)	1.24 (0.77, 2.00)	0.37	404	41 (9.2)	11.4 (8.2, 15.7)	1.05 (0.64, 1.75)	0.85

[a] Associations between lipoproteins and baseline prevalence of major PAD were tested in the whole cohort using logistic regression models, adjusting for sex, age, duration of diabetes, BMI, systolic and diastolic BP, HbA_{1c}, urinary ACR (using a natural log transformation), eGFR, history of tobacco smoking (never, former, current), history of diabetic retinopathy or macrovascular disease and use of antihypertensive treatment, statin, fibrate, antiplatelet or anticoagulant drug and metformin

[b] Associations between lipoproteins and incident PAD were tested in participants without a baseline history of PAD. The 10 year cumulative incidences, with associated 95% CIs, were estimated using the Kaplan–Meier survival analyses. HR, with associated 95% CIs, were computed using Cox proportional hazards regression models adjusting for age, sex, duration of diabetes, BMI, systolic BP, urinary ACR, eGFR, history of tobacco smoking (never, former, current), history of diabetic retinopathy and use of antihypertensive treatment, statin, metformin and insulin therapy

$p<0.05$ was significant

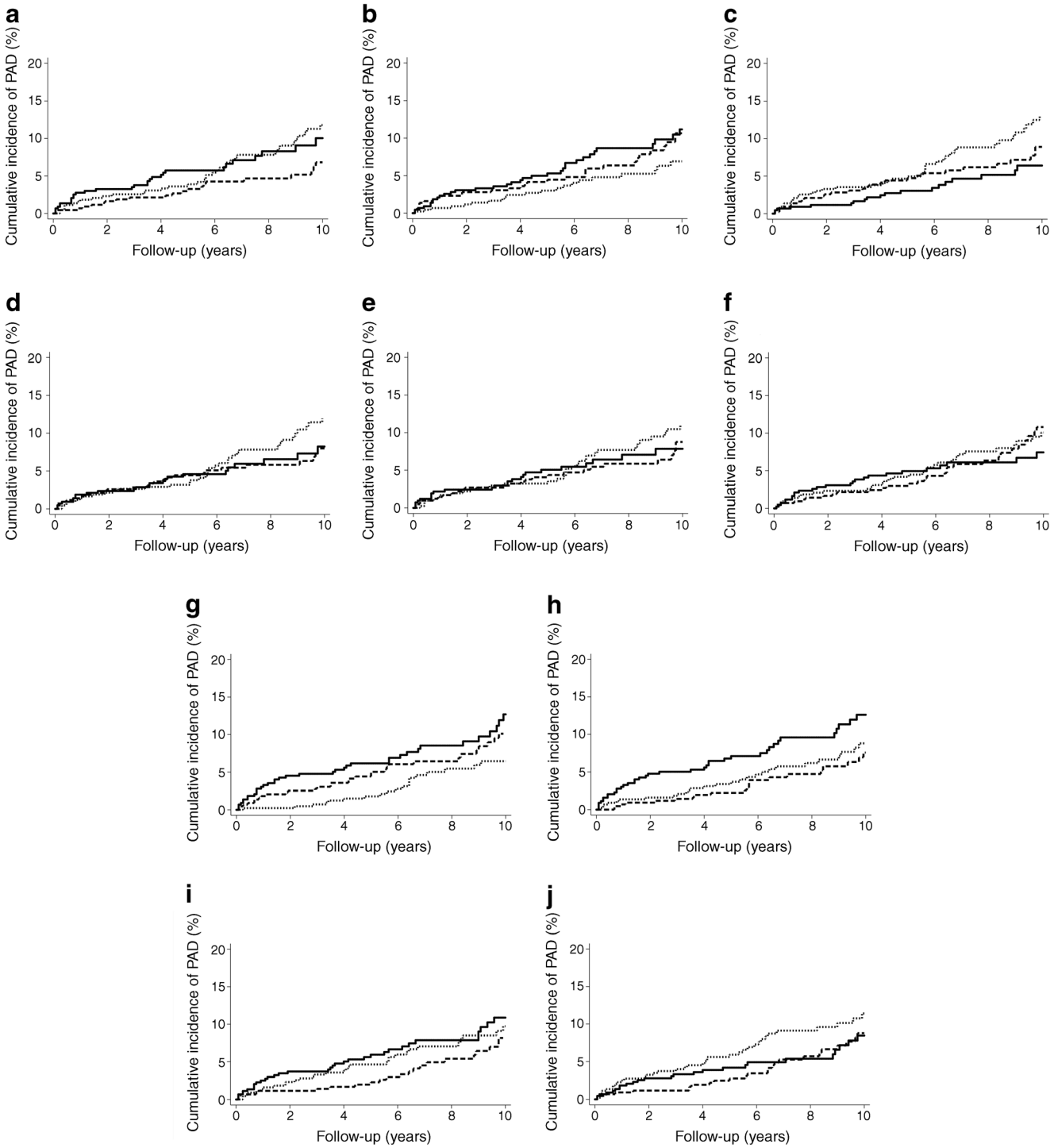

Fig. 1 Cumulative incidence of major PAD during follow-up according to the first (solid line), second (dashed line) and third tertiles (dotted line) of plasma concentrations of total cholesterol (**a**, p=0.26), HDL-cholesterol (**b**, p=0.05), total cholesterol/HDL-cholesterol ratio (**c**, p=0.03), non-HDL-cholesterol (**d**, p=0.41), LDL-cholesterol (**e**, p=0.68), triacylglycerol (**f**, p=0.96), ApoA-I (**g**, p=0.009), ApoA-II (**h**, p=0.02), ApoB-100 (**i**, p=0.43) and Lp(a) (**j**, p=0.13). p values provided from logrank test comparing the three tertiles for each lipid variable. Analyses performed in 1339 participants without a history of PAD at baseline

in the highest, compared with those in the lowest, tertiles of HDL-cholesterol and ApoA-I. In addition, the prevalence of major PAD increased in the upper tertiles of total cholesterol/HDL-cholesterol ratio, compared with the lowest tertiles, and

Table 3 Incidences of lower-limb amputation and revascularisation during follow-up by plasma concentrations of lipoproteins at baseline

Variable	Lower-limb amputation during follow-up[a]					Lower-limb arterial revascularisation during follow-up[b]				
	No, *n*	Yes, *n* (%)	10 year cumulative incidence, % (95% CI)[c]	HR (95% CI)[d]	*p* value	No, *n*	Yes, *n* (%)	10 year cumulative incidence, % (95% CI)[c]	HR (95% CI)[d]	*p* value
Total cholesterol										
First tertile	446	19 (4.1)	5.9 (3.5, 9.7)	Reference		440	25 (5.4)	8.0 (5.2, 12.2)	Reference	
Second tertile	448	17 (3.7)	4.3 (2.4, 7.3)	1.00 (0.50, 1.98)	0.99	446	19 (4.1)	4.4 (2.6, 7.3)	0.93 (0.49, 1.72)	0.81
Third tertile	446	19 (4.1)	4.2 (2.5, 6.9)	0.95 (0.48, 1.89)	0.88	431	34 (7.3)	10.4 (7.2, 14.6)	1.31 (0.74, 2.35)	0.36
HDL-cholesterol										
First tertile	440	22 (4.8)	5.5 (3.4, 8.7)	Reference		431	31 (6.7)	9.9 (6.6, 14.3)	Reference	
Second tertile	440	23 (5.0)	6.3 (4.0, 10.0)	1.15 (0.63, 2.13)	0.64	434	28 (6.1)	7.5 (4.9, 11.3)	0.89 (0.52, 1.50)	0.66
Third tertile	454	8 (1.7)	1.8 (1.0, 3.8)	0.35 (0.14, 0.81)	0.01	444	19 (4.1)	6.4 (4.0, 10.0)	0.52 (0.27, 0.97)	0.04
Total cholesterol/HDL-cholesterol ratio										
First tertile	450	12 (2.6)	3.1 (1.7, 5.6)	Reference		444	18 (3.9)	6.0 (3.6, 9.6)	Reference	
Second tertile	444	19 (4.1)	4.9 (2.9, 8.2)	1.65 (0.80, 3.56)	0.18	437	25 (5.4)	6.1 (3.9, 9.4)	1.73 (0.97, 3.15)	0.06
Third tertile	440	22 (4.8)	5.4 (3.4, 8.5)	2.11 (1.02, 4.56)	0.04	428	35 (7.6)	11.5 (7.5, 16.1)	4.34 (1.98, 9.22)	0.0004
Non-HDL-cholesterol										
First tertile	448	14 (3.0)	3.7 (2.2, 6.3)	Reference		441	21 (4.5)	6.7 (4.2, 10.4)	Reference	
Second tertile	443	19 (4.1)	4.9 (2.9, 8.2)	1.33 (0.65, 2.76)	0.43	437	25 (5.4)	6.2 (4.0, 9.5)	1.18 (0.65, 2.18)	0.58
Third tertile	443	20 (4.3)	4.6 (2.8, 7.5)	1.49 (0.72, 3.16)	0.28	431	32 (6.9)	10.1 (6.7, 14.3)	1.51 (0.83, 2.80)	0.18
LDL-cholesterol										
First tertile	432	14 (3.1)	4.1 (2.4, 6.9)	Reference		426	21 (4.7)	6.6 (4.2, 10.4)	Reference	
Second tertile	427	19 (4.3)	5.1 (3.0, 8.4)	1.37 (0.67, 2.85)	0.39	423	24 (5.4)	6.7 (4.3, 10.3)	1.18 (0.64, 2.19)	0.59
Third tertile	428	19 (4.3)	4.7 (2.9, 7.7)	1.37 (0.65, 2.92)	0.40	417	30 (6.7)	9.1 (6.2, 13.1)	1.40 (0.76, 2.61)	0.28
Triacylglycerol										
First tertile	447	16 (3.5)	4.0 (2.3, 7.0)	Reference		443	20 (4.3)	4.7 (2.9, 7.7)	Reference	
Second tertile	445	18 (3.9)	5.3 (3.1, 8.8)	1.51 (0.76, 3.05)	0.24	435	28 (6.1)	9.6 (6.0, 13.9)	1.41 (0.78, 2.57)	0.25
Third tertile	445	19 (4.1)	4.3 (2.6, 6.9)	1.49 (0.75, 2.99)	0.25	434	30 (6.5)	8.9 (6.1, 12.8)	1.61 (0.90, 2.94)	0.11
ApoA-I										
First tertile	441	23 (5.0)	6.6 (4.1, 10.4)	Reference		432	32 (6.9)	10.3 (6.8, 15.0)	Reference	
Second tertile	443	22 (4.7)	4.9 (3.0, 7.7)	1.03 (0.56, 1.89)	0.93	437	27 (5.8)	8.5 (5.6, 12.6)	0.86 (0.50, 1.45)	0.56
Third tertile	455	9 (1.9)	2.6 (1.3–5.2)	0.43 (0.18, 0.92)	0.03	446	19 (4.1)	5.2 (3.2, 8.4)	0.53 (0.28, 0.96)	0.04
ApoA-II										
First tertile	441	23 (5.0)	6.0 (3.8, 9.5)	Reference		433	31 (6.7)	9.7 (6.5, 14.1)	Reference	
Second tertile	447	17 (3.7)	4.3 (2.5, 7.3)	0.86 (0.44, 1.65)	0.65	446	18 (3.9)	6.0 (3.6, 9.7)	0.60 (0.32, 1.08)	0.09
Third tertile	451	14 (3.0)	3.6 (2.0, 6.3)	0.81 (0.39, 1.62)	0.55	436	29 (6.2)	8.1 (5.5, 11.8)	1.29 (0.75, 2.22)	0.36
ApoB-100										
First tertile	446	18 (3.9)	4.4 (2.7, 7.0)	Reference		436	28 (6.0)	8.9 (5.9, 13.2)	Reference	
Second tertile	445	19 (4.1)	5.0 (2.9, 8.4)	1.32 (0.68, 2.57)	0.40	443	21 (4.5)	6.0 (3.7, 9.6)	0.83 (0.46, 1.48)	0.53
Third tertile	448	17 (3.7)	4.3 (2.5, 7.2)	1.35 (0.68, 2.67)	0.39	436	29 (6.2)	8.8 (6.0, 12.6)	1.36 (0.78, 2.35)	0.27

the incidence of major PAD increased in the upper tertiles of both total cholesterol/HDL-cholesterol ratio and non-HDL-cholesterol. These associations were independent on putative confounding variables including key cardiovascular risk factors and were mainly reliable after treating all-cause death and coronary events as competing risks. Comparable results were observed when we considered lower-limb amputation and requirement of revascularisation individually as secondary endpoints.

Few studies have examined the relationship between lipoproteins and PAD in people with type 2 diabetes and results have been contrasting. Reduced HDL-cholesterol

Table 3 (continued)

Variable	Lower-limb amputation during follow-up[a]					Lower-limb arterial revascularisation during follow-up[b]				
	No, *n*	Yes, *n* (%)	10 year cumulative incidence, % (95% CI)[c]	HR (95% CI)[d]	*p* value	No, *n*	Yes, *n* (%)	10 year cumulative incidence, % (95% CI)[c]	HR (95% CI)[d]	*p* value
Lp(a)										
First tertile	447	17 (3.7)	4.7 (2.8, 7.9)	Reference		448	16 (3.4)	5.4 (3.1, 9.2)	Reference	
Second tertile	446	18 (3.9)	4.9 (2.9, 8.0)	0.77 (0.39, 1.54)	0.46	441	23 (5.0)	7.1 (4.6, 10.8)	1.11 (0.58, 2.16)	0.75
Third tertile	446	19 (4.1)	4.1 (2.5, 6.8)	0.75 (0.37, 1.52)	0.43	426	39 (8.4)	11.0 (7.8, 15.2)	1.60 (0.90, 2.99)	0.11

[a] Analyses were performed in participants without a baseline history of lower-limb amputation

[b] Analyses were performed in participants without a baseline history of lower-limb revascularisation

[c] The 10 year cumulative incidences, with associated 95% CIs, were estimated using the Kaplan–Meier survival analyses

[d] HRs, with associated 95% CIs, were computed using Cox proportional hazards regression models, for lower-limb amputation or revascularisation in the second and third tertiles vs the first tertile (reference) of plasma concentrations of lipoproteins, adjusting for sex, age, duration of diabetes, BMI, systolic BP, urinary ACR, eGFR, history of tobacco smoking (never, former, current), history of diabetic retinopathy and use of antihypertensive treatment, statin, metformin and insulin therapy

$p<0.05$ was significant

concentration was an independent risk factor for PAD in the UK Prospective Diabetes Study (UKPDS) [16]. In contrast, no independent association was observed between lipid variables and PAD in the Action in Diabetes and Vascular Disease: PreterAx and DiamicroN Modified-Release Controlled Evaluation (ADVANCE) and the Bypass Angioplasty Revascularisation Investigation in Type 2 Diabetes (BARI-2D) studies [17, 18]. The lipoproteins' profiles (decreased HDL-cholesterol and increased LDL-cholesterol and atherogenic lipids) seem to be more consistent in the general population with PAD than in people with diabetes [19–21]. The definitions of PAD were comparable in studies of diabetic and non-diabetic individuals, varying from abnormal ankle–brachial index to symptomatic PAD including intermittent claudication and requirement of revascularisation [16–21]. The principal difference between the two populations could be that amputations and microvascular disease are more common in individuals with diabetes [3, 17], albeit the involvement of microvascular disease in PAD was also reported in individuals without diabetes [22].

Overall, the inverse association between plasma HDL-cholesterol and the risk of CVD is among the most consistent and reproducible associations in epidemiological studies [23–25] but whether this association is causal remains unclear. The most recognised function of HDL lipoproteins is reverse cholesterol transport, leading to the removal of excess cholesterol from peripheral tissues to the liver. The uptake and transport of cholesterol by HDL for hepatic excretion prevents and potentially reverses its peripheral accumulation in arteries [26–28]. HDL particles have been also associated with some other valuable effects, including antioxidative, antithrombotic, anti-inflammatory and vasodilatory functions [29]. Normalisation of HDL-cholesterol has been described as an unmet need in the management of patients with high cardiovascular risk, including type 2 diabetes [30], but Mendelian randomisation studies have generated scepticism about the hypothetical HDL causality [31, 32]. Furthermore, randomised clinical trials showed that drugs increasing plasma HDL-cholesterol did not reduce the risk of atherosclerotic CVD. Cholesteryl ester transfer protein (CETP) inhibitors have either had negative, neutral or only minor beneficial effects on cardiovascular outcomes despite substantial increase in HDL-cholesterol levels [33–36]. The paradoxical inability of HDL-cholesterol-raising therapies to reduce cardiovascular adverse events may be explained by a highly complex and multifunctional biology of the HDL lipoprotein system. A recent study has shown significant associations between dysfunctional HDL particles and increased risk of acute coronary syndrome and its manifestations in individuals at high cardiovascular risk [37]. Treatments targeting HDL functions could be a potential therapeutic approach. Meanwhile, HDL-cholesterol measurement remains a key component of CVD risk stratification and is still recommended as such [38].

As far as we know, this is the first investigation of the relationship between plasma concentrations of apolipoproteins and the risk of major PAD in a prospective cohort of people with type 2 diabetes. Class A apolipoproteins are the major structural and functional protein components of HDL; they stabilise HDL lipoprotein structure, solubilise their lipid component and help in reverse cholesterol transport. They also act as ligands for cellular receptor binding and enzyme

activators or inhibitors. ApoA-I accounts for approximately 70% of HDL structure while ApoA-II corresponds to about 20%. Our findings pointed out an independent and reliable association between high ApoA-I concentrations and reduced risk of major PAD. Plasma concentrations of ApoA-II were also inversely associated with a greater prevalence of PAD at baseline and increased risk of incident PAD during follow-up but the latter association was not consistent and was mainly dependent on confounding variables. We did not observe evidence for significant interaction between HLD-cholesterol and ApoA-I in their association with the risk of major PAD, suggesting that these lipid variables may interact differently on this condition, although our data cannot allow any mechanistic conclusion. ApoA-I has also been linked to CVD [25, 37]; a large meta-analysis emphasised not only an inverse association between ApoA-I and a reduction of major cardiovascular events but also showed that increase in ApoA-I concentrations led to decreased cardiovascular risk among statin-treated patients [25].

We did not observe significant association between triacylglycerol, ApoB-100 or Lp(a) and major PAD. Also, plasma concentrations of LDL-cholesterol were not significantly associated with increased risk of major PAD during follow-up ($p = 0.05$). The fact that LDL-cholesterol was not measured in our cohort but was only estimated using the Friedewald formula after excluding participants with high levels of triacylglycerol may have mitigated our results. Nevertheless, our findings are consistent with those of a recent prospective study reporting a significant association between low standard plasma concentration of HDL-cholesterol (but not LDL-cholesterol) and incident PAD events among women without known CVD at baseline [21]. However, this study showed strong associations between excess incident PAD and a series of atherogenic lipidomic features: reduced HDL; and elevated LDL particles, small LDL particles and medium and very large VLDL particles. Of note, we have observed an increased risk of incident major PAD in the top tertile of non-HDL-cholesterol, which estimates total concentrations of all atherogenic ApoB-containing lipoproteins including triacylglycerol-rich particles in VLDLs and their remnants. This association was independent of confounders without evidence for significant interaction with HDL-cholesterol, ApoA-I or use of statins. However, this association did not persist after treating all-cause death or coronary events as competing risks, although having a similar magnitude to the association observed in the primary analyses. In addition, plasma concentrations of non-HDL-cholesterol was not associated with prevalent PAD. This difference cannot be explained by the baseline characteristics of participants in the prevalent and the incident PAD groups, as these were roughly comparable. A potential explanation for this difference is that incident PAD seemed to be more likely related to large-vessel disease than prevalent PAD. Indeed, the requirement of revascularisation accounted for 72% of incident PAD while limb loss was more frequent at baseline (5.0% vs 3.9% at the time of endpoint), including mainly minor amputation (74% at baseline vs 45% at the time of endpoint). Additionally, arterial stenosis with significant haemodynamic effects was observed in 95% of amputees at the time of endpoint (vs 59% at baseline). Taken together, these findings suggest that increased non-HDL-cholesterol could mainly reflect a high risk of macrovascular disease in patients with PAD. Non-HDL-C has been suggested as a pragmatic and cost-effective cardiovascular biomarker, especially in people with type 2 diabetes [38, 39].

Our study also highlights the total cholesterol/HDL-cholesterol ratio as a strong and consistent lipid biomarker for the risk of major PAD. A high total cholesterol/HDL-cholesterol ratio was associated with both prevalent and incident PAD. This association was independent of relevant confounders, persisted when we dealt with all-cause death or coronary events as competing risk, and was also reliable when we considered incident lower-limb amputation and revascularisation individually. These findings are consistent with those of an earlier study reporting total cholesterol/HDL-cholesterol ratio as a strong and independent predictor of PAD in a nested case–control cohort from the Physicians' Health Study [20].

The key strength of our work is the investigation of a prospective cohort of individuals with type 2 diabetes collecting a wide range of clinical and biological features at baseline with adjudicated outcomes during a median follow-up of 7 years. We have measured a set of lipid and apolipoprotein compounds, which may reflect at least partly a factual picture of lipoproteins' profile in individuals with type 2 diabetes. However, our study may not be representative of all populations with type 2 diabetes as SURDIAGENE is a French mono-centre inpatient cohort. Also, 45% participants were on statin therapy at baseline, which may influence our findings as this treatment reduces the risk of PAD events [40]. However, all our analyses were adjusted for statin use and we did not observe significant interaction between statin use and relevant lipids biomarkers in their relationship with major PAD. On the other hand, we assessed only baseline use of statin, leaving some uncertainty about potential increase in statin use during follow-up that may possibly bias our results. The issue is that we cannot assess time-varying hazards as we do not have data regarding the use of statins over time. Our investigation may also omit potential association between lipid variables and early stages of PAD as we have evaluated only advanced PAD-related events. Finally, we did not have accurate and comprehensive data regarding peripheral neuropathy. Of note, all amputees had a strong evidence of PAD (abolition of peripheral pulses, intermittent claudication or lower-limb artery stenosis with haemodynamic effects). At the same time, peripheral diabetic neuropathy and foot infection were reported in 51–76% of amputees, supporting the notion that lower-limb amputation is a dramatic consequence

of several concomitant complications including microvascular, macrovascular and infectious disease.

In conclusion, we have observed independent and reliable associations between plasma concentrations of HDL-cholesterol, total cholesterol/HDL-cholesterol ratio and ApoA-I and the prevalence at baseline and the incidence during follow-up of major PAD in individuals with type 2 diabetes. Increased non-HDL-cholesterol concentrations were also associated with increased incidence of major PAD. Our findings may help to identify a specific lipoprotein's profile in individuals with type 2 diabetes at high risk of major PAD.

Supplementary Information The online version contains peer-reviewed but unedited supplementary material available at https://doi.org/10.1007/s00125-020-05326-x.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Contribution statement CB, PJS, SH and KM designed the study and researched data. CB and KM drafted the manuscript. PJS and SH contributed to discussion and reviewed/edited the manuscript. MC and VB researched data, contributed to discussion and reviewed/edited the manuscript. LP, EG, SR, FS, OB, LBB, GV, MM, RR and VR participated in the analyses and the interpretation of data and contributed to discussion and reviewed/edited the manuscript. All authors approved the current version of the manuscript. KM is the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses.

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