The relation between VLDL-cholesterol and risk of cardiovascular events in patients with manifest cardiovascular disease

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ABSTRACT

Introduction: Apolipoprotein B containing lipoproteins are atherogenic. There is evidence that with low plasma low density lipoprotein cholesterol (LDL-C) levels residual vascular risk might be caused by triglyceride rich lipoproteins such as very-low density lipoproteins (VLDL), chylomicrons and their remnants. We investigated the relationship between VLDL-cholesterol (VLDL-C) and recurrent major adverse cardiovascular events (MACE), major adverse limb events (MALE) and all-cause mortality in a cohort of patients with cardiovascular disease.
Methods: Prospective cohort study in 8057 patients with cardiovascular disease from the UCC-SMART study. The relation between calculated VLDL-C levels and the occurrence of MACE, MALE and all-cause mortality was analyzed with Cox regression models.
Results: Patients mean age was 60 ± 10 years, 74% were male, 4894 (61%) had coronary artery disease, 2445 (30%) stroke, 1425 (18%) peripheral arterial disease and 684 (8%) patients had an abdominal aorta aneurysm at baseline. A total of 1535 MACE, 571 MALE and 1792 deaths were observed during a median follow up of 8.2 years (interquartile range 4.512.2). VLDL-C was not associated with risk of MACE or all-cause mortality. In the highest quartile of VLDL-C the risk was higher for major adverse limb events (MALE) (HR 1.49; 95%CI 1.16–1.93) compared to the lowest quartile, after adjustment for confounders including LDL-C and lipid lowering medication.
Conclusion: In patients with clinically manifest cardiovascular disease plasma VLDL-C confers an increased risk for MALE, but not for MACE and all-cause mortality, independent of established risk factors including LDL-C and lipid-lowering medication.

1. Introduction

Apolipoprotein B (ApoB) containing lipoproteins are atherogenic and contribute to the development cardiovascular disease [1–4]. ApoB containing lipoproteins include chylomicrons, very low density lipoproteins (VLDL), their remnants, and low density lipoproteins (LDL). Historically, the main focus has been on LDL-cholesterol (LDL-C) plasma levels in both risk prediction and as treatment target [5]. However, ApoB containing lipoproteins only consist of approximately 60% LDL-C. In recent years, non-high density lipoprotein cholesterol (non-HDL-C) has been increasingly studied as risk predictor and as an alternative treatment target, especially in patients with (mild) hypertriglyceridemia [5]. Non-HDL-C reflects cholesterol in all ApoB containing lipoproteins and is calculated as total cholesterol (TC) minus HDL—C. Previous studies have shown that non-HDL-C is a better predictor of cardiovascular events than LDL-C and some guidelines therefore recommend using non-HDL-C in addition to LDL-C as treatment target [6,7]. In a fasting state, non-HDL-C levels contain LDL-C and VLDL-cholesterol (VLDL-C), including VLDL-remnants.

Remnants are the smaller residues of VLDL that remain after lipolysis of triglycerides (TG) as a result of lipoprotein lipase (LPL) activity. An easy approach to estimate VLDL-C levels is subtracting HDL-C and LDL-C from TC in a fasting state, since chylomicrons are only present in plasma postprandial. Together with chylomicrons and chylomicron-remnants, VLDL and VLDL-remnants are also often called triglyceride rich lipoproteins (TRLs). Of these, chylomicron- and VLDL-remnants

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are particularly atherogenic because of their reduced size and relatively high cholesterol content in addition to pro-inflammatory properties due to their triglyceride content [8]. These are small enough to enter the vascular wall where they get trapped in the intima, causing foam cell accumulation and low-grade inflammation, both contributing to the development of atherosclerosis [4,9].

In patients with coronary artery disease (CAD) it is shown that TRLs are associated with cardiovascular disease [3,10,11]. A study in 10.001 patients with CAD receiving atorvastatin 10 mg showed that increased fasting calculated remnant cholesterol (VLDL-C) levels were associated with an increased risk of MACE for the highest VLDL-C quintile versus the lowest quintile [3]. Previous studies have shown that TRLs are associated with increased risk for cardiovascular events in the general population [12–15], in patients with Familial Hypercholesterolemia (FH) [16], in patients with type 2 diabetes mellitus (T2DM) and in patients with chronic kidney disease (CKD) [17].

This raised the question whether VLDL-C is a risk factor for recurrent vascular disease and whether this effect is independent from LDL-C and lipid-lowering therapy in patients with clinical manifest vascular disease. Therefore, the aim of the present study is to establish the association between calculated VLDL-C and risk of major adverse cardiovascular events (MACE) major adverse limb events (MALE), the separate components of MACE (myocardial infarction (MI), stroke and cardiovascular mortality) and all-cause mortality in a cohort of patients with different clinical manifestations of arterial vascular disease.

2. Methods

2.1. Study design and patients

The Utrecht Cardiovascular Cohort - Secondary Manifestations of ARTerial disease (UCC-SMART) study is an ongoing, single-center, dynamic, prospective cohort of patients aged 18 to 80 referred to the University Medical Center Utrecht (UMC Utrecht) in the Netherlands, for management of cardiovascular risk factors or atherosclerotic cardiovascular disease. The study was approved by the Ethics Committee of the UMC Utrecht and all patients gave their written informed consent. The rationale and design has been published previously [18].

For the present study, we used data of 8139 patients, enrolled in the UCC-SMART study between September 1996 and March 2017, with a history or recent diagnosis of clinically manifest arterial disease, including coronary artery disease (CAD), cerebrovascular disease (CeVD), peripheral artery disease (PAD) and/or aneurysm of the abdominal aorta (AAA). CAD was defined as either diagnosis of myocardial infarction (MI), angina pectoris or coronary stenosis in ≥1 major coronary artery, or self-reported history of MI, cardiac arrest or revascularization. CeVD was defined as either diagnosis of transient ischemic attack, ischemic stroke, amaurosis fugax or retinal infarction, or self-reported stroke or carotid artery operation in the past. PAD was defined as Fontaine stage of at least IIa (i.e. intermittent claudicatio and resting ankle-brachial index (ABI) <0.9 in at least one leg), or a self-reported history of amputation or vascular surgery of the lower extremities. AAA was defined as an aneurysm of the abdominal aorta (distal aortic diameter ≥ 3 cm) during screening or AAA surgery in the past. Patients could be classified in more than one vascular disease category at baseline. Patients with TG levels >9 mmol/l were excluded because in these patients LDL-C cannot reliably be estimated using the Friedewald formula ($n = 23$) [19]. In addition, known homozygotes of ApoƐ2 genotype were excluded ($n = 59$) since some of these patients might have familial dysbetalipoproteinemia (FD) and LDL-C cannot be accurately calculated in these patients [20]. In total, the cohort consisted of 8057 patients.

2.2. Screening at baseline

At baseline all patient characteristics were determined using a standardized screening protocol consisting of questionnaires, physical examination, laboratory testing, ankle-brachial index, and abdominal aortic and carotid ultrasound.

TC, HDL-C and TG were measured using enzymatic colorimetric methods (AU5811 analysers, Beckman and Coulter). ApoB measurements were included from 2006 onwards and measured using a nephelometer. LDL-C was calculated using the Friedewald formula up to a plasma TG level of 9 mmol/l [19,20]. VLDL-C was calculated as fasting TC minus LDL-C minus HDL—C.

T2DM was defined as a referral diagnosis of T2DM, self-reported use of glucose-lowering agents or insulin or fasting plasma glucose level ≥ 7.0 mmol/l at screening in combination with receiving glucose-lowering therapy within 1 year from screening. Medication use was self-reported. Lipid-lowering medication included use of statins, fibrates, bile acid sequestrants or nicotinic acid at baseline. Prescription of high intensity statins was defined as atorvastatin ≥40 mg or rosuvastatin ≥20 mg. Alcohol use was defined as self-reported current or recently stopped alcohol consumption and no alcohol use was defined as past or never alcohol consumption. Metabolic syndrome was defined according to the Adult Treatment Panel (ATP) III criteria [21] as having 3 or more of the following criteria: waist circumference (WC) >102 cm for males and > 88 cm for females; TG ≥1.7 mmol/l; HDL-C < 1.03 mmol/l for males and HDL-C < 1.29 for females, systolic blood pressure (SBP) ≥130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg; fasting plasma glucose ≥5.6 mmol/l.

2.3. Follow-up

The incidence of recurrent cardiovascular events was evaluated bi-annually in all patients with a questionnaire to obtain information about outpatient clinic visits and hospitalizations. Whenever a possible event was reported, all relevant data were collected. All events were evaluated by three independent physicians of the UCC-SMART Study Endpoint Committee. The primary outcome for this study was MACE, defined as non-fatal MI, non-fatal stroke and cardiovascular mortality. Secondary outcomes were MALE (major amputation or lower limb revascularization), the separate components of MACE: MI, stroke and cardiovascular mortality; and all-cause mortality. For detailed definitions of outcomes see supplementary table 1. Follow-up was defined as time between date of inclusion and the date of first cardiovascular event, death from any cause, lost to follow-up ($n = 469$), or end of follow-up in March 2017.

2.4. Data analyses

Patient characteristics are presented stratified in quartiles for VLDL-C. In the baseline table (Table 1) continuous variables are shown as mean with standard deviation (SD) or median with interquartile range (IQR) in case of a skewed distribution. Categorical variables are shown as number with percentage. Cox proportional hazard models were used to calculate hazard ratios (HR) and corresponding 95% confidence intervals (95%CI) in quartiles with the lowest quartile serving as reference (Table 2) for the occurrence of vascular events. When a patient had multiple events, the first recorded event was used in the analyses. Patients were censored if they were lost to follow-up or if they died. Potential confounders were selected prior to the analyses based on causal diagrams. Two models were built, model I was adjusted for age and sex and model II was additionally adjusted for LDL-C, current smoking, waist circumference, creatinine level, systolic blood pressure, T2DM and use of lipid-lowering medication. Also, in exploratory analyses additional adjustments for HbA1c, HOMA-IR, HDL—C, hsCRP or alcohol use were performed.

Table 1
Baseline characteristics according to VLDL-C quartiles.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Total	P-value
VLDL-C (range, mmol/l)	0.09–0.45	0.46–0.63	0.64–0.90	0.91–4.01	0.09–4.01	
VLDL-C (median, mmol/l)	0.36	0.54	0.76	1.21	0.63	
	n = 2210	n = 2085	n = 1882	n = 1880	n = 8057	
Male	1592 (72%)	1512 (73%)	1394 (74%)	1450 (77%)	5948 (74%)	0.001
Age (years)	60 ± 11	61 ± 10	60 ± 10	57 ± 10	60 ± 10	<0.001
BMI (kg/m^2)	25.6 ± 3.6	26.6 ± 4.0	27.5 ± 4.1	28.1 ± 4.1	26.9 ± 4.0	<0.001
Waist circumference (cm)	92 ± 12	95 ± 12	98 ± 12	100 ± 12	96 ± 12	<0.001
Systolic blood pressure (mmHg)	137 ± 20	139 ± 21	140 ± 21	142 ± 21	139 ± 21	<0.001
Diastolic blood pressure (mmHg)	80 ± 11	81 ± 11	81 ± 12	82 ± 11	81 ± 11	<0.001
Current smoker	555 (25%)	608 (29%)	589 (31%)	736 (39%)	2488 (31%)	<0.001
Pack years[1]	11 (0–27)	14 (2–30)	16 (3–32)	23 (7–36)	14.6 (2.7–31.5)	<0.001
Use of alcohol in the last year	1462 (66%)	1220 (59%)	944 (50%)	834 (45%)	4460 (56%)	<0.001
>20 units of alcohol every week	213 (10%)	184 (9%)	181 (10%)	253 (13%)	831 (10%)	<0.001
Medical history						
CAD	1287 (58%)	1277 (61%)	1174 (62%)	1156 (61%)	4894 (61%)	0.035
CeVD	763 (35%)	606 (29%)	555 (29%)	521 (28%)	2445 (30%)	<0.001
PAD	291 (13%)	339 (16%)	348 (18%)	447 (24%)	1425 (18%)	<0.001
AAA	149 (7%)	185 (9%)	170 (9%)	180 (10%)	684 (8%)	0.005
T2DM	247 (11%)	287 (14%)	348 (18%)	463 (25%)	1345 (17%)	<0.001
Metabolic syndrome	541 (25%)	767 (37%)	1218 (65%)	1679 (90%)	4205 (52%)	<0.001
Medication						
Lipid-lowering medication	1625 (74%)	1430 (69%)	1251 (66%)	1192 (63%)	5498 (68%)	<0.001
- Statins	1617 (73%)	1413 (68%)	1236 (66%)	1176 (63%)	5442 (68%)	<0.001
- Fibrates	7 (0%)	11 (1%)	17 (1%)	26 (2%)	61 (1%)	<0.001
- Bile acid sequestrant	1 (0%)	2 (0%)	3 (0%)	3 (0%)	9 (0%)	0.36
- Ezetemibe	77 (4%)	67 (3%)	56 (3%)	61 (4%)	261 (3%)	0.95
High intensity statins	187 (8%)	152 (7%)	137 (7%)	154 (8%)	630 (8%)	0.36
Blood pressure lowering medication	1559 (71%)	1558 (75%)	1436 (76%)	1456 (77%)	6009 (75%)	<0.001
Platelet aggregation inhibitors	1791 (81%)	1618 (78%)	1408 (75%)	1364 (73%)	6181 (77%)	<0.001
Laboratory measurements						
Total cholesterol (mmol/l)	4.3 ± 1.0	4.7 ± 1.1	5.0 ± 1.1	5.4 ± 1.2	4.8 ± 1.2	<0.001
HDL-C (mmol/l)	1.4 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	1.2 ± 0.4	<0.001
LDL-C (mmol/l)*	2.5 ± 0.9	2.9 ± 1.0	3.0 ± 1.0	3.0 ± 1.1	2.8 ± 1.0	<0.001
Non-HDL-C (mmol/l)	2.9 ± 0.9	3.4 ± 1.0	3.8 ± 1.0	4.4 ± 1.2	3.6 ± 1.2	<0.001
Triglycerides (mmol/l)[1]	0.8 (0.7–0.9)	1.2 (1.1–1.3)	1.7 (1.5–1.8)	2.7 (2.3–3.3)	1.4 (1.0–2.0)	<0.001
ApoB (g/l)[‡]	0.7 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	<0.001
HOMA-IR[1]	2.0 (1.3–2.9)	2.5 (1.7–3.8)	3.0 (2.0–4.6)	3.9 (2.5–6.3)	2.6 (1.7–4.2)	<0.001
HsCRP (mg/l)[1]	1.5 (0.7–3.3)	1.9 (0.9–4.2)	2.2 (1.1–4.8)	2.7 (1.4–5.5)	2.0 (1.0–4.4)	<0.001
eGFR (CKD-EPI, ml/min/1.73m^2)	79 ± 16	77 ± 17	76 ± 18	76 ± 20	77 ± 18	<0.001

Abbreviations: VLDL-C = very-low density lipoprotein cholesterol; BMI = Body Mass Index; CAD = coronary artery disease; CeVD = cerebrovascular disease; PAD = peripheral artery disease; AAA = abdominal aortic aneurysm; T2DM = type 2 diabetes mellitus; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; non-HDL = non-high-density lipoprotein cholesterol; ApoB = Apolipoprotein B; HOMA-IR = Homeostasis Model Assessment – Insuline Resistance; HsCRP = high sensitivity C-reactive protein; eGFR = estimated glomerular filtration rate (calculated with Chronic Kidney Disease Epidemiology Collaboration [CKDEPI] formula).

* Calculated with Friedewald formula up to triglycerides of 9 mmol/l.

[1] Median with interquartile range.

[‡] Measured from January 2006.

Linearity assumption was tested visually and statistically by adding continuous VLDL-C level as a restricted cubic spline function to the model (MACE p for linearity 0.92 and MALE p for linearity 0.22). The proportional hazard assumption, examined graphically by plotting scaled Schoenfeld residuals against time, was not violated. Formal testing of the PH assumption confirmed this with a *p*-value of 0.56.

Cumulative incidence plots derived from a Kaplan-Meier curve were made for the incidence of MACE and MALE (Fig. 1) and a histogram of the distribution of VLDL-C in the total population and in patients with and without metabolic syndrome was made (supplementary fig. 1).

We tested for interaction of VLDL-C with LDL-C and use of lipid-lowering medication for MACE and MALE and stratified for LDL-C levels above and below 1.8 mmol/l according to secondary prevention guidelines [5] and use of lipid-lowering medication (supplementary table 2). In addition, we stratified for type of vascular disease (i.e. CAD, CeVD, PAD and AAA) at baseline (supplementary table 3). Single imputation was performed by bootstrapping and predictive mean matching, based on multiple regression to account for missing data. Missing values ranged from 0.21% for systolic blood pressure to 12.3% for waist

circumference. For all analyses, a *p*-value of <0.05 was considered significant. R Studio, version 3.5.1, was used for the statistical analyses.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the study population are presented according to quartiles of calculated VLDL-C as well as for the total study population in Table 1. In total, 74% of the cohort were males, and mean age was 60 (SD 10.3) years, 61% had a history of CAD, 30% of CeVD, 18% of PAD and 8% of AAA. Furthermore, 17% of the patients had T2DM and 52% metabolic syndrome. In higher quartiles of VLDL-C, the prevalence of the metabolic syndrome was higher i.e. 25% in the lowest quartile compared to 90% in the highest quartile. Patients in the highest quartile had higher TC, LDL-C, non-HDL-C, TG, ApoB and hsCRP concentrations as compared to patients in the lowest VLDL-C quartile. The use of statins was 73% in the lowest quartile and 63% in the highest quartile. The distribution of VLDL-C in the total population

Table 2
Risk of vascular events according to VLDL-C quartiles.

	Model	Quartile 1 n = 2210 HR (95% CI)	Quartile 2 n = 20853 HR (95% CI)	Quartile 3 n = 1882 HR (95% CI)	Quartile 4 n = 1880 HR (95% CI)
Major adverse cardiovascular events (MACE)	# events	322	377	399	437
	I	Reference	1.12 (0.97–1.30)	1.27 (1.09–1.47)*	1.48 (1.27–1.70)*
	II	Reference	1.00 (0.86–1.16)	1.04 (0.89–1.21)	1.10 (0.94–1.28)
Major adverse limb events (MALE)	# events	102	126	150	193
	I	Reference	1.23 (0.95–1.60)	1.58 (1.23–2.03)*	2.11 (1.66–2.69)*
	II	Reference	1.10 (0.85–1.44)	1.31 (1.01–1.70)*	1.49 (1.16–1.93)*
Myocardial infarction (MI)	# events	94	126	156	183
	I	Reference	1.29 (0.99–1.68)	1.68 (1.30–2.17)*	1.94 (1.51–2.50)*
	II	Reference	1.19 (0.91–1.56)	1.47 (1.12–1.92)*	1.64 (1.26–2.14)*
Stroke	# events	104	105	109	113
	I	Reference	0.98 (0.75–1.29)	1.09 (0.83–1.43)	1.20 (0.91–1.57)
	II	Reference	0.87 (0.66–1.15)	0.91 (0.69–1.21)	0.93 (0.69–1.23)
Cardiovascular mortality	# events	94	222	235	259
	I	Reference	1.16 (0.95–1.41)	1.31 (1.07–1.59)*	1.60 (1.32–1.94)*
	II	Reference	0.99 (0.81–1.21)	0.99 (0.81–1.21)	1.06 (0.87–1.30)
All-cause mortality	# events	396	437	472	487
	I	Reference	1.03 (0.89–1.17)	1.16 (1.02–1.33)*	1.30 (1.14–1.49)*
	II	Reference	0.90 (0.79–1.04)	0.94 (0.82–1.09)	0.95 (0.82–1.09)

Model I: age + sex.
Model II: Model 1 + LDL-C + smoking + waist circumference + creatinine + systolic blood pressure + T2DM + use of lipid lowering medication.
 * $P < .05$.

and in patients with and without metabolic syndrome is shown in supplementary fig. 1.

3.2. VLDL-C and risk of recurrent vascular events

A total of 1535 first MACE were observed, of which 559 were myocardial infarctions (MI), 431 strokes, 897 cardiovascular deaths. Furthermore, there were 571 MALE and all-cause mortality was 1792 during a total follow-up of 68.699 person-years with median follow-up of 8.2 (IQR 4.5–12.2) years.

Overall, in the highest VLDL-C quartile the HR was 1.49 (95%CI 1.16–1.93) for MALE and HR 1.64 (95%CI 1.26–2.14) for MI compared to the lowest VLDL-C quartile (Table 2). The risk for MACE, stroke, cardiovascular- and all-cause mortality was not significantly different in the highest compared to the lowest quartile. Exploratory analysis with additional adjustment for HbA1c, HOMA-IR, HDL—C, hsCRP or alcohol use did not change the results.

There was no effect modification by LDL-C or the use of lipid-lowering medication on the relationship between VLDL-C and vascular outcomes. The *p*-value for interaction of LDL-C was 0.50 for MACE and 0.09 for MALE (i.e. both no significant interaction) and for use of lipid-lowering medication the p's were 0.32 and 0.77 for MACE and MALE respectively.

Despite absence of effect modification by LDL-C levels and use of lipid-lowering medication, we evaluated the risk for recurrent events stratified for LDL-C treatment targets according to guidelines [5] and use of lipid-lowering medication (supplementary table 2). Although not reaching statistical significance, the risk for MALE in the group with low LDL-C levels was similar to the group with high LDL-C levels (HR 1.33 95%CI 0.97–1.82 versus HR 1.26 95%CI 1.05–1.52).

Furthermore, we showed that even with use of lipid-lowering medication VLDL-C is a risk factor for MALE (HR 1.39 95%CI 1.13–1.72) and MI (HR 1.44 95%CI 1.20–1.73), but not for the other outcomes. In addition, we evaluated the risk of recurrent vascular events according to location of vascular disease at baseline (supplementary table 3). In patients with CAD a 1 mmol/l increase in VLDL-C was related to increased risk of MACE (HR 1.19, 95%CI 1.04–1.37), MALE (HR 1.30, 95% CI 1.03–1.65) and MI (HR 1.31, 95%CI 1.07–1.59). In patients with CeVD at baseline a 1 mmol/l higher VLDL-C was associated with an increased risk for MALE (HR 1.72, 95%CI 1.23–2.39) and MI (HR 1.68,

95%CI 1.20–2.35). Levels of VLDL-C in patients with PAD were not associated with other vascular outcomes including MALE. In a subgroup of 684 patients with AAA, VLDL-C was associated with incident MALE (HR 1.80, 95%CI 1.22–2.64) but not with other vascular outcomes.

4. Discussion

The present study shows that VLDL-C is associated with an increased risk of MALE, but not with MACE and all-cause mortality, independent of LDL-C and lipid-lowering medication in patients with cardiovascular disease.

A post hoc analysis of the TNT trial (in patients with CAD using atorvastatin 10 mg) showed that patients in the highest quintile of fasting calculated remnant cholesterol (VLDL-C) have a higher risk of MACE (composite of CHD death, nonfatal non–procedure-related myocardial infarction, resuscitated cardiac arrest, or fatal or nonfatal stroke) compared with patients in the lowest quintile (HR: 1.48 95%CI 1.15–1.92), independent of LDL-C levels [3]. We found no relation between high levels of VLDL-C and MACE, probably due to limited power since the continuous analyses showed a significant effect of VLDL-C on MACE (data not shown). A recent study showed that directly measured TRL-C is in particular associated with PAD in women from the general population [15]. A case control study in men with and without PAD also showed that remnant abnormalities play an important role in the development and severity of PAD [22] and another study showed that chylomicron- and VLDL-remnants are significantly increased in patients with intermittent limb claudication compared to controls [23]. The present study also showed a strong relationship between VLDL-C and the development of MALE. This relationship is most likely predominantly caused by VLDL-remnant cholesterol and the results indicate that remnant cholesterol might be a specific risk factor for the development of PAD. Furthermore, a study in patients with ischemic heart disease showed that patients in the highest tertile of calculated nonfasting remnant cholesterol (including chylomicrons and chylomicron remnants) have an increased risk of all-cause mortality compared to the lowest tertile (HR 1.3, 95%CI 1.2–1.5) [24]. These results are in contrast to the present study, however, these analyses were not adjusted for LDL-C levels and prescription of lipid-lowering medication.

Regarding the separate components of MACE, VLDL-C was only associated with an increased risk of MI, and no relation for stroke or
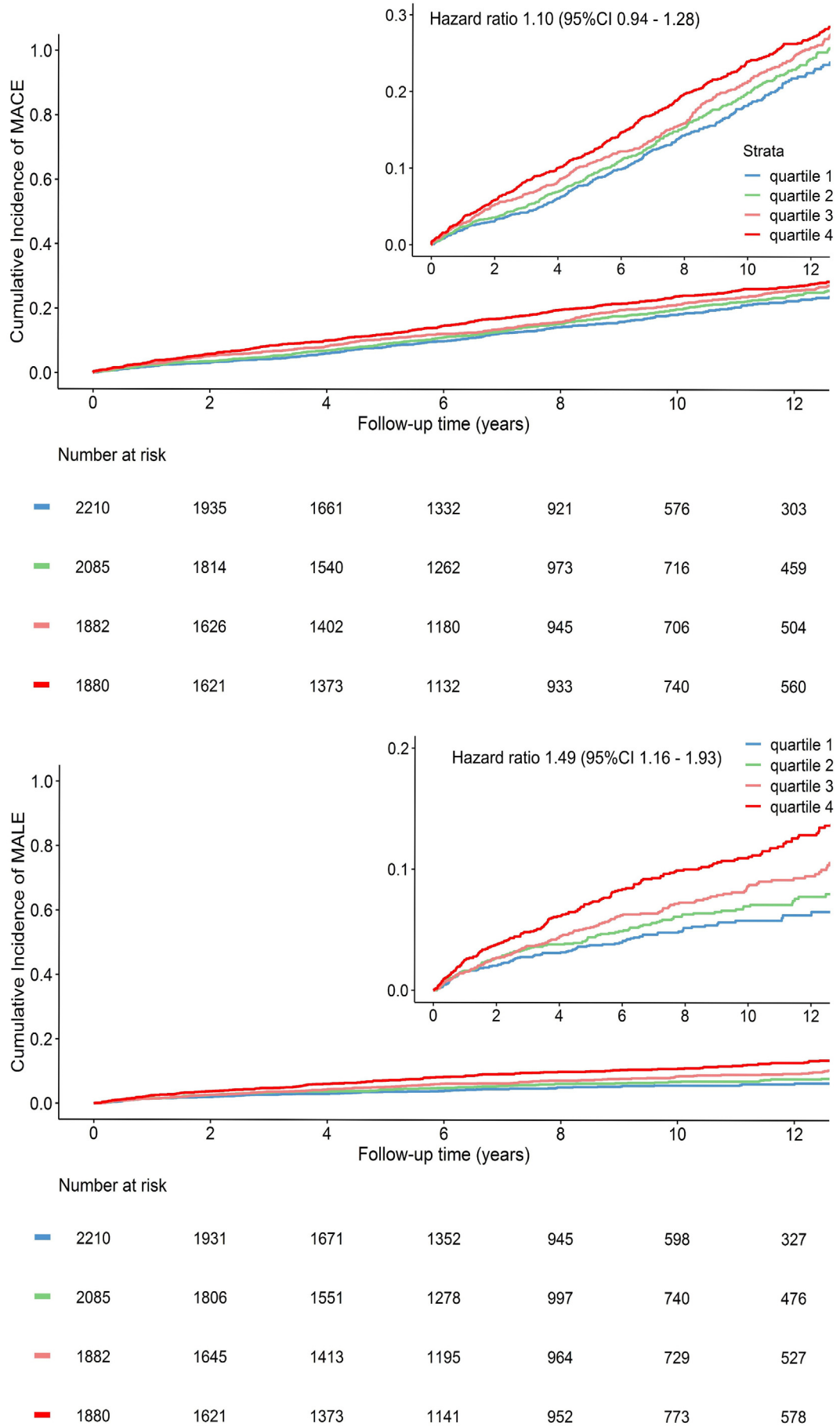
Fig. 1. Cumulative incidence of MACE (A) and MALE (B) among quartiles of VLDL-C.

cardiovascular mortality was observed. These results are in line with previous research which have shown that remnant cholesterol is a causal risk factor for CAD [25,26]. However, in contrast to the present study, research in population based cohorts also showed an increased risk for ischemic stroke [27] and all-cause mortality [14]. This difference could be due to differences in medication use or length of follow-up in the different study populations.

In line with previous research, this study showed that in patients with CAD, VLDL-C was related to a higher risk of recurrent cardiovascular events. This was not only due to the relatively large sample size of patients with CAD compared to other subgroups (and therefore reaching statistically significance more rapidly), the effect estimates of the hazard ratios are also higher in the CAD group compared to patients in other subgroups. This was also shown in a cohort of 560 patients with CAD and low LDL-C levels on lipid-lowering medication [28]. Hence, VLDL-C might attribute to the residual cardiovascular risk in patients with CAD. However, in the present study there was no association with MALE in patients with a history of PAD, which is possibly explained by index-event bias.

The formula to calculate VLDL-C (TC – HDL-C – LDL-C) is commonly used to give an estimation of cholesterol in VLDL in a fasting state. Several studies [14,24,27] use the formula to estimate VLDL-C in the non-fasting state where the calculated lipoprotein fraction also consists of chylomicrons and their remnants in addition to VLDL(remnants). The pro-atherogenic nature of the VLDL-C subfraction does not only depend on the cholesterol concentration but also on the size of particles, with smaller particles (VLDL-remnants) being more atherogenic than larger particles (VLDL). This means that atherogenicity of total VLDL-C may differ according to the proportion of VLDL-remnants. Similarly to LDL-C, cholesterol in remnant lipoproteins becomes trapped in the intima [4], Unlike LDL-C, cholesterol in remnant lipoproteins does not require oxidation to be absorbed by macrophages [29]. Remnant lipoproteins are relatively cholesterol rich compared to larger TRLs due to lipolysis, and contain more cholesterol per particle compared to LDL particles [2]. Therefore, remnant lipoproteins can cause serious foam cell accumulation. On top of this, remnant lipoproteins are also associated with inflammation, where LDL-C is not [9]. A possible explanation for this is that hydrolysis fof triglycerides in TRLs will generate inflammation due to the release of free fatty acids that induce local endothelial inflammation [30]. In line with this, we found increasing levels of hsCRP across quartiles of remnant cholesterol (Table 1), indicating a higher level of inflammation with higher levels of VLDL-C.

In line with previous studies [3,11,28,31] this study showed that VLDL-C remains a risk factor for recurrent cardiovascular events, even when patients with vascular disease use lipid-lowering medication or achieve LDL-C treatment goals (supplementary table 2). This underlines the need for therapies specifically intervening with VLDL-C and TRL metabolism. Several new therapies are currently evaluated in clinical studies. Apolipoprotein C3 (ApoC3) is present on TRLs and promotes the assembly and secretion of TRLs [32] and inhibits LPL and hepatic lipase [33]. Loss-of-function ApoC3 mutations are associated with a reduced incidence of cardiovascular disease, an association mainly mediated by decreased remnant cholesterol levels [34]. In patients with Familial Chylomicronemia Syndrome it was shown that volanesorsen, an antisense oligonucleotide for ApoC3, lowered VLDL-C with 58% [35]. Angiopoietin-like protein 3 (ANGPTL3) reversibly inhibits LPL activity and is mainly active in the postprandial phase [36]. Loss-of-function mutations of ANGPTL3 are related to a decreased incidence of coronary artery disease and both antisense oligonucleotides for ANGPTL3 and a monoclonal antibody for ANGPTL3, evinacumab, have been shown to reduce TG by approximately 60% [37].

Strengths of this study are the prospective study design, a large number of patients with different locations of vascular disease, and the long follow-up period and number of endpoints. Furthermore, calculated VLDL-C can easily be calculated from a conventional lipid panel and is therefore clinically available. Some study limitations should be considered. First, LDL-C levels were estimated with the Friedewald formula which uses a standard proportion of cholesterol versus triglycerides (5 triglycerides for 1 cholesterol molecule) to estimate LDL-C. Therefore VLDL-C is an approximation and not an absolute measurement. This could lead to a less precise estimation of VLDL-C. To address this we excluded all patients in which the Friedewald formula was not valid. Furthermore, VLDL-C consists of VLDL and VLDL-remnants and we were not able to evaluate the precise distribution of cholesterol in these lipoproteins. Second, plasma lipids were measured only once at baseline, so we could not account for natural variation or variation as a result of initiating lipid-lowering medication during the follow-up period. As the cohort started in 1996 only 68% of the patients in this cohort were prescribed statins at baseline, which could lead to an underestimation of the true risk for cardiovascular disease. Third, *APOE* genotyping was not available for the complete cohort (two third of the cohort was genotyped), possibly causing an incomplete exclusion of patients with an homozygous ApoƐ2 genotype.

In conclusion, in patients with clinically manifest cardiovascular disease plasma VLDL-C confers an increased risk for MALE, but not for MACE and all-cause mortality, independent of LDL-C and lipid-lowering medication. We therefore suggest to use also non-HDL-C in clinical practice and to pay attention to VLDL-C in patients who develop a vascular event despite low LDL-C levels or use of lipid-lowering medication.

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Author contributions

All authors contributed to either the acquisition, analysis, or interpretation of the data for the work.

All authors have given final approval of the manuscript, and agree to be accountable for the work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2020.08.030.

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