

Triglyceride-Rich Lipoprotein and LDL Particle Subfractions and Their Association With Incident Type 2 Diabetes: The PREVEND Study.

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Abstract

Background: Triglyceride-rich lipoproteins particles (TRLP) and low density lipoprotein particles (LDLP) vary in size. Their association with β -cell function is not well described. We determined associations of TRLP and LDLP subfractions with β -cell function, and evaluated their associations with incident T2D in the general population.

Methods: We included 4818 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study without T2D at baseline. TRLP and LDLP subfraction concentrations and their average sizes were measured using the LP4 algorithm of the Vantera nuclear magnetic resonance platform. HOMA-IR was used as measure of insulin resistance. HOMA- β was used as a proxy of β -cell function.

Results: In subjects without T2D at baseline, very large TRLP, large LDLP and LDL size were inversely associated with HOMA- β , whereas large TRLP, medium TRLP and TRL size were positively associated with HOMA- β taking account of HOMA-IR. During a median follow-up of 7.3 years, 263 individuals developed T2D. In multivariable-adjusted Cox regression models, higher concentrations of total, very large, large, and very small TRLP and TRL size were associated with an increased T2D risk after adjustment for relevant covariates, including age, sex, BMI, HDL-C, HOMA- β , and HOMA-IR. On the contrary, higher concentrations of large LDLP and LDL size were associated with a lower risk of developing T2D.

Conclusions: Specific TRL and LDL particle characteristics are associated with β -cell function taking account of HOMA-IR. Moreover, TRL and LDL particle characteristics are differently associated with incident T2D, even when taking account of HOMA- β and HOMA-IR.

Background

The prevalence of type 2 diabetes (T2D) is rapidly increasing worldwide with an estimated global prevalence of 548 million people in 2045 [1]. It is known for decades that T2D is featured by abnormalities in circulating lipoproteins, comprising elevations in triglyceride-rich apolipoprotein B (apoB)-containing lipoproteins and low levels of high density lipoproteins (HDL) [2–4]. Thus, T2D confers elevated levels of large sized very low-density lipoproteins (VLDL) and smaller sized low-density lipoproteins (LDL) [5, 6]. Interestingly, recent studies have suggested that abnormalities in triglyceride-rich apolipoprotein B (apoB)-containing lipoproteins may precede deterioration in insulin resistance [7–12], which in turn is a major risk factor for T2D development [13–15]. Nonetheless, while exaggerated deposition of cholesterol in the pancreatic β cell is likely to adversely affect in insulin secretion [16–20], little information is available on the role of circulating VLDL and LDL and their subfractions in β cell failure [16, 21].

Recently, a nuclear magnetic resonance (NMR) spectroscopy-based method was developed for quantifying triglyceride-rich lipoprotein (TRL) and LDL particle and subfraction concentrations in human plasma [22]. Using NMR methodology it has been suggested that large VLDL particles and VLDL size as

well as small LDL particles are positively associated with incident T2D. By contrast, concentrations of small VLDL and large LDL particles as well as LDL size were inversely associated with incident T2D, independent of established risk factors including glucose and insulin resistance [23–25]. Although evidence is mounting that HDL cholesterol and HDL subfractions predict incident T2D even independent of homeostasis model assessment of insulin resistance (HOMA-IR) [26], the association of TRL and LDL particle and subfraction concentrations with β -cell function, estimated as homeostasis model assessment of β -cell function (HOMA- β) have not been determined in a population-based cohort so far.

The aims of the present study were i) to cross-sectionally explore the associations between TRL and LDL particle and subfraction concentrations with HOMA β taking account of insulin resistance and ii) to longitudinally determine the associations of TRL and LDL particle and subfraction concentrations with incident T2D. To this end NMR-derived lipoproteins we measured in the population-based Prevention of Renal and Vascular End-Stage Disease (PREVEND) study.

Methods

Study design and participants

The study was performed among participants of the Prevention of RENal and Vascular ENd-stage Disease (PREVEND) cohort study. Details of the study design and recruitment have been described elsewhere [27]. Briefly, all residents of Groningen aged 28 to 75 years were invited to participate in this study from 1997 to 1998. Pregnant women, individuals with type 1 diabetes and T2D using insulin were excluded. Participants with a urinary Albumin excretion UAE ≥ 10 mg/L ($n = 6000$) and control group with a UAE < 10 mg/L ($n = 2,595$), a total of 8592 individuals, completed an extensive first screening. A second screening round took place from 2001 to 2003, encompassing 6,894 participants and was considered the “baseline” for the current study. Individuals with missing data on diabetes and follow-up and those with missing NMR or covariate data at baseline were excluded, leaving 308 subjects with pre-existing diabetes and 4818 subjects without diabetes at baseline of the present study.

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The protocol for the PREVEND study was approved by the local ethics committee of the University Medical Center Groningen (approval number: MEC96/01/022). All subjects included in the present analysis provided written informed consent to participate, and the study procedures were conducted according to the Declaration of Helsinki.

Clinical and laboratory measurement

During two outpatient clinic visits separated by 3 weeks, baseline data on demographics, lifestyle parameters, cardiovascular and renal disease history, smoking habits and medication use were collected.

During two outpatient clinic visits separated by 3 weeks, baseline data on demographics, lifestyle parameters, cardiovascular and renal disease history, smoking habits and medication use were collected. Smoking and alcohol use were based on self-reports. Self-reported alcohol consumption was recorded in as : absence (no alcohol consumption), 1 to 4 units/month, 2 to 7 units/week, 1 to 3 units/day, or > 3 units/day; 1 unit was considered to contain 10 grams of alcohol [28]. We categorized alcohol consumption as high intake > 3 units/day vs. the rest (no high intake \leq 3 units/day. Information on medication use was combined with information from IADB.nl, a database containing information on prescribed medication in public pharmacies in The Netherlands since 1999 (<http://www.iadb.nl/>). Height and weight were measured with the participants standing without shoes or heavy outer garments. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Systolic and diastolic blood pressure values were recorded as the means of the last 2 recordings of the second visit.

Plasma samples were taken from participants after an overnight fast, were prepared by centrifugation at 4°C and stored at – 80°C until analysis. Fasting plasma glucose (FPG) was measured by dry chemistry (Eastman Kodak, Rochester, NY, USA). EDTA-anticoagulated plasma samples were stored at – 80 °C. Insulin was measured with an immunoturbidometric assay (Diazyme Laboratories, Poway, CA, USA). HOMA-IR was calculated as fasting plasma insulin (mU/L) \times fasting plasma glucose (mmol/L)/22.5. HOMA- β was calculated $20 \times$ fasting plasma insulin (mU/L)/[fasting plasma glucose (mmol/L)-3.5] [29]. High sensitivity C-reactive protein (hs-CRP) was assayed by nephelometry (Dade Behring Diagnostic, Marburg, Germany). Urinary albumin was measured by nephelometry (Dade Behring Diagnostic, Marburg, Germany). Serum creatinine was determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, New York) and serum cystatin C level by nephelometry (BN II N) (Dade Behring Diagnostic, Marburg, Germany). Estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation.

Lipoprotein parameters were measured by *NMR LipoProfile*® testing at Labcorp (Morrisville, NC). NMR spectra were collected on an optimized version of the NMR LipoProfile test (LP4 algorithm) [22, 26, 30]. Very large, large, medium, small, and very small triglyceride rich lipoprotein particles (TRL) and large, medium, and small LDL particles (LDL) were quantified using the conventional deconvolution method and the amplitudes of their spectroscopically distinct lipid methyl group NMR signals [31]. Total TRL were calculated as the sum of the concentrations of very large, large, medium, small, and very small TRL. Total LDL were calculated as the sum of the concentrations of large, medium, and small LDL. Mean TRL and LDL size were calculated using the weighted averages derived from the sum of the diameters of each subfraction. Estimated ranges of particle diameter for the TRL and LDL subfractions were as follows: very large TRL, 90–240 nm; large TRL, 50–89 nm; medium TRL, 37–49 nm; small TRL, 30–36 nm; very small TRL, 24–29 nm; large LDL, 21.5–23 nm; medium LDL, 20.5–21.4 nm; and small LDL, 19–20.4 nm. Total cholesterol (TC), triglycerides, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and apoB using equations derived from lipoprotein measures assayed on a Vantera Clinical NMR Analyzer platform using Partial Least-Squares (PLS) regression models. Non-HDL cholesterol was calculated as the difference between TC and HDL-C.

Outcome ascertainment

Follow-up time was defined as the period between the baseline measurement (second screening) and the date of ascertainment of T2D. In case a person moved to an unknown destination, the census date was the date of removal from the municipal registry. T2D was ascertained if one or more of the following criteria were met: 1) FPG ≥ 7.0 mmol/L (126 mg/dL), 2) random sample plasma glucose ≥ 11.1 mmol/L (200 mg/dL), 3) self-report of a physician diagnosis of T2D, and 4) initiation of glucose-lowering medication use, retrieved from a central pharmacy registry.

Statistical analyses

All analyses were conducted using the statistical packages IBM SPSS (version 24.0.1; SPSS, Chicago, IL, USA) and STATA/SE (version 14; StataCorp, College Station, TX, USA). For all analyses, 2-sided P values < 0.05 were considered statistically significant. Results were expressed as mean \pm standard deviation (SD), median with interquartile range (IQR) or number (percentage) for normally distributed, skewed and categorical data, respectively. Baseline characteristics are presented for the whole cohort and according to T2D status (pre-existing T2D at baseline, developed T2D and did not develop T2D during follow-up time). Differences in baseline characteristics between the 3 groups were tested using one-way ANOVA or Kruskal Wallis tests for skewed continuous variables with subsequent Bonferroni correction, and χ^2 tests for categorical variables. Univariate Pearson correlation coefficients were estimated to assess the cross-sectional correlations of non-HDL cholesterol, LDL-C, triglycerides, apoB, TRL and LDL subfractions and size with HOMA-IR and HOMA- β in subjects without T2D and with T2D at baseline, separately. Multivariable linear regression models were adjusted for sex, age, and HOMA-IR or HOMA- β (model 1); plus BMI, lipid-lowering medication, anti-hypertensive medication (model 2a) for subjects without T2D; plus glucose-lowering medication for subjects with pre-existing T2D (model 2b). Variables with a skewed distribution were \log_e transformed. Crude and multivariable Cox proportional hazards regression analyses were performed to examine the associations between each predictor and incident T2D. In addition, hazards ratios (HR) with 95% confidence intervals (CIs) were calculated per 1 SD increment of predictors (\log_e transformed for variables which were not normally distributed). The following five cumulative models were used for adjustment: age and sex (models 1); plus alcohol use, BMI, family history of diabetes, lipid-lowering medication and anti-hypertensive medication (models 2); plus HDL-C (models 3a) or HDL size (models 3b); plus HOMA- β (models 4a and 4b); plus HOMA-IR (models 5a and 5b). Interactions were tested to assess statistical evidence for effect modification by using statins or high alcohol use in crude analyses. For predictors of which the interaction term was significant, we performed subgroup analyses.

Results

Baseline characteristics

308 individuals had pre-existing T2D at baseline, 263 subjects developed T2D during follow-up of 7.3 (6.1–7.7) years and 4555 subjects did not develop T2D during follow-up (Table 1). Individuals who developed T2D were more likely to be men and to have a family history of diabetes more frequently than participants who did not develop T2D. Subjects with pre-existing T2D were older and consumed less alcohol in comparison with two other groups without T2D at baseline. Individuals with pre-existing T2D and who developed T2D had a higher BMI, Systolic blood pressure (SBP), FPG, HOMA-IR, hs-CRP but lower eGFR, and they used anti-hypertensive and lipid-lowering medication more frequently than individuals who did not develop T2D. HOMA- β was lower in the subjects with pre-existing T2D. While TC was significantly higher in subjects developed T2D compared to the subjects with pre-existing T2D, non-HDL cholesterol was significantly higher in who developed T2D compared to the subjects who did not develop T2D. Triglycerides, apoB, total TRLP, TRLP subfractions and size (except small TRLP), as well as total LDLP and small LDLP were higher, whereas HDL-C, medium and large LDLP and LDL size were lower in individuals with pre-existing T2D and those who developed T2D compared to participants who did not develop T2D.

Table 1
Baseline characteristics of subjects with pre-existing diabetes, who developed and who did not develop T2D.

Diabetes at baseline		Incident diabetes		
Variables		Yes	No	P value
Participants, n	308	263	4555	
General characteristics				
Female, %	43.8	38.0	51.2	< 0.001 ^a b c
Age, year	62.6 ± 9.9	57.0 ± 9.7	52.5 ± 11.6	< 0.001 a b c
Lifestyle parameters				
Current smoker, %	20.1	28.1	26.8	0.626
Alcohol consumption,				0.082
None, %	39.8	26.7	23.0	0.032
1–4 units per month, %	15.1	14.5	17.4	
2–7 units per week, %	24.3	28.2	32.4	
1–3 units per day, %	17.1	23.7	23.1	
>3 units per day, %	3.6	6.8	4.1	
Family history of Diabetes %	31.5	33.1	16.6	< 0.001 b c
Body composition				
Weight, kg	87.7 ± 16.5	88.6 ± 15.3	78.7 ± 14.0	< 0.001 a b
Height, cm	171.1 ± 10.0	172.5 ± 9.2	173.2 ± 9.4	0.001 ^a
BMI, kg/m ²	29.9 ± 5.2	29.8 ± 4.7	26.2 ± 4.0	< 0.001 a b
Waist circumference, cm	101.9 ± 12.7	101.7 ± 12.6	90.6 ± 12.1	< 0.001 a b
Blood pressure				
Systolic blood pressure, mmHg	135.3 ± 19.8	135.9 ± 19.7	124.3 ± 17.7	< 0.001 a b

Diabetes at baseline		Incident diabetes		
Diastolic blood pressure, mmHg	75.3 ± 9.0	77.3 ± 9.0	72.9 ± 8.9	< 0.001 a b c
Hypertension, %	63.6	55.5	25.8	< 0.001 a b c
Anti-hypertensive medication, %	44.8	35.1	14.0	< 0.001 a b c
Lipid-lowering medication, %	26.3	19.0	7.3	< 0.001 a b c
Statin use, %	23.8	16.1	6.6	< 0.001 a b c
Glucose homeostasis				
FPG, mmol/L	8.1 ± 2.4	5.7 ± 0.7	4.8 ± 0.6	< 0.001 a b c
Insulin, mU/L	14.3 (9.5–21.4)	13.6(9.2–20.3)	7.7 (5.6–11.3)	< 0.001 a b
HOMA-IR, (mU/L ²)/22.5	4.9 (3.1–7.9)	3.4 (2.3–5.4)	1.6 (1.1–2.4)	< 0.001 a b c
HOMA-β, %	68.7 (42.1-113.6)	131.1 (84.4-202.2)	133.3 (91.7-203.3)	< 0.001 a c
Hs-CRP, mg/L	2.6 (1.3–4.9)	2.1 (1.1–3.8)	1.2 (0.5–2.8)	< 0.001 a b
Renal function				
eGFR, mL/min per 1.73 m ²	85.9 (73.0-98.9)	90.0 (79.0-100.5)	95.0 (83.0-105.0)	< 0.001 a b c
Urinary Albumin excretion, mg/24 h	16.3 (8.2–46.0)	12.2 (7.8–30.0)	8.4 (6.0-14.4)	< 0.001 ^a b c
Lipids and lipoproteins				
Total cholesterol, mg/dL	190.6 ± 40.2	198.5 ± 36.2	193.5 ± 34.4	0.023 ^c
Non-HDL cholesterol, mg/dL	146.0 ± 38.4	152.4 ± 35.6	141.5 ± 35.7	< 0.001 ^b
LDL-C, mg/dL	112.7 ± 32.6	118.2 ± 30.0	113.9 ± 29.1	0.050
HDL-C, mg/dL	44.6 ± 9.9	45.9 ± 9.9	52.0 ± 12.2	< 0.001 a b

Diabetes at baseline		Incident diabetes		
Triglycerides (total), mg/dL	127.7 (88.7-179.1)	134.6 (89.2-198.9)	91.0 (65.1-133.9)	< 0.001 ^a b
ApoB, mg/dL	93.9 ± 24.7	97.6 ± 23.2	90.2 ± 23.1	< 0.001 ^a b
TRLP, nmol/L	164.0 (126.1–212.0)	176.9 (132.3–220.0)	147.4 (109.2-189.7)	< 0.001 a b
Very large TRLP, nmol/L	0.06 (0.01–0.20)	0.06 (0.01–0.27)	0.04 (0.01–0.09)	< 0.001 a b
Large TRLP, nmol/L	4.8 (1.9–9.3)	5.5 (2.4–10.8)	1.7 (0.2-5.0)	< 0.001 ^a b
Medium TRLP, nmol/L	17.3 (7.5–31.7)	18.2 (8.0-32.2)	11.3 (5.4–21.3)	< 0.001 a b
Small TRLP, nmol/L	48.5 (22.4–84.3)	47.3 (24.3–84.3)	46.8 (25.2–79.4)	0.073
Very small TRLP, nmol/L	77.5 (40.0-118.5)	86.8 (44.4-130.8)	68.8 (37.2–108.0)	0.006 ^b
TRL size, nm	50.0 ± 9.2	51.3 ± 9.9	45.5 ± 8.2	< 0.001 a b
LDLP, nmol/L	1567.9 (1252.7–1833.0)	1611.6 (1343.7-1895.6)	1467.3 (1226.1-1727.3)	< 0.001 a b
Large LDLP, nmol/L	198.1 (55.2-373.7)	244.2 (54.4-414.5)	378.9 (197.0-567.1)	< 0.001 a b
Medium LDLP, nmol/L	300.5 (39.8-677.4)	362.6 (72.8-755.7)	386.6 (126.3–723.0)	< 0.001 a b
Small LDLP, nmol/L	843.2 (508.1-1214.7)	821.1 (519.2-1194.7)	533.8 (305.9-838.6)	< 0.001 a b
LDL size, nm	20.7 ± 0.6	20.8 ± 0.6	21.1 ± 0.5	< 0.001 a b

Diabetes at baseline	Incident diabetes
<p>Data are the mean \pm SD, median (interquartile range) unless otherwise indicated. Significance was tested by one-way ANOVA tests and Kruskal Wallis tests where appropriate. ^aDifferent between the group with T2D at baseline and the group without T2D at follow up. ^bDifferent between the group with incident T2D and the group without T2D at follow up. ^cDifferent between the group with T2D at baseline and the group with incident T2D at follow up at p value < 0.05 (by Bonferroni correction). BMI: Body mass index; hs-CRP: high sensitivity C-reactive protein ; FPG: fasting plasma glucose; HOMA-IR: Homeostatic model assessment of insulin resistance; HOMA-β : Homeostatic model assessment of β-cell function; eGFR: estimated glomerular filtration rate; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; ApoB: apolipoprotein B; LDLP: low density lipoprotein particles; TRLP: triglyceride rich lipoprotein particles.</p>	

In subjects 1) with pre-existing T2D, 2) those who developed T2D and 3) those did not develop T2D during follow-up, lipids and lipoprotein values were compared between participants using a statin and those who did not (Additional file1: Table S1). Overall, TC, non-HDL-C, LDL-C, apoB, small TRLP, total LDLP, large and medium LDLP and LDL size were lower in statin users compared to non-statin users in each group.

Furthermore, lipids and lipoprotein values were compared between participants who consumed high alcohol and those who did not consume high alcohol in subjects with pre-existing T2D, those who developed T2D and those did not develop T2D during follow-up (Additional file1: Table S2). TC, non-HDL-C, LDL-C, triglycerides, apoB, total, very large, large and very small TRLP, TRL size, and small LDLP where higher, whereas small TRLP and medium LDLP where lower in subjects with high alcohol intake compared to subject who did not consume high amounts of alcohol particularly in those subjects who did not develop T2D during follow-up.

Cross-sectional associations

Univariable and multivariable analyses were performed to show the association between lipid and lipoprotein measures with HOMA-IR (Table 2) and HOMA- β (Table 3). In the group with T2D at baseline, triglycerides, very large and large TRLP and TRL size, as well as total and small LDLP, were positively, whereas large LDLP and LDL size were inversely associated with HOMA-IR in the univariate model and in the multivariable models adjusted for age, sex, HOMA- β , BMI and medication use (Table 2). In the group without T2D at baseline, non-HDL cholesterol, LDL-C, triglycerides, apoB, TRLP subfractions (except small and very small TRLP) and size, as well as total and small LDLP were positively, whereas large LDLP and LDL size were inversely associated with HOMA-IR in the univariate model and in multivariable models (Table 2).

Table 2

Correlations between lipids, TRL and LDL particle concentrations, subfractions and sizes with HOMA-IR

Variables	T2D at baseline (n = 308), std β , P-values			No T2D at baseline (n = 4818), std β , P-values		
	Univariate	Model 1	Model 2b	Univariate	Model 1	Model 2a
Non-HDL cholesterol	0.099	0.094	0.065	0.229***	0.181***	0.143***
LDL-C	0.046	0.019	0.036	0.149***	0.110***	0.083***
Triglycerides	0.274***	0.329***	0.303***	0.402***	0.349***	0.279***
ApoB	0.102	0.098	0.072	0.246***	0.192***	0.153***
TRLP	0.090	0.103	0.105	0.237***	0.187***	0.143***
Very large TRLP	0.267***	0.332***	0.284***	0.228***	0.256***	0.214***
Large TRLP	0.329***	0.380***	0.330***	0.385***	0.345***	0.266***
Medium TRLP	0.055	0.093	0.137*	0.254***	0.207***	0.159***
Small TRLP	-0.058	-0.100	-0.082	-0.004	-0.017	0.008
Very small TRLP	0.066	0.092	0.147*	0.015	-0.005	-0.020
TRL size	0.349***	0.403***	0.345***	0.315***	0.294***	0.208***
LDLP	0.158**	0.160**	0.131*	0.238***	0.184***	0.142***
Large LDLP	-0.197***	-0.254***	-0.258***	-0.252***	-0.228***	-0.195***
Medium LDLP	-0.056	-0.094	-0.106	-0.047***	-0.067***	-0.035
Small LDLP	0.243***	0.286***	0.297***	0.135***	0.120***	0.086***
LDL size	-0.232***	-0.332***	-0.357***	-0.271***	-0.236***	-0.195***

Standardized β coefficients (Std β) are shown. Log_e transformed values are used for variables with a skewed distribution.

Model 1: sex, age, and HOMA- β

Model 2a: model 1 + BMI, lipid-lowering medication, anti-hypertensive medication

Model 2b: model 1 + BMI, lipid-lowering medication, anti-hypertensive medication, and anti-diabetic medication

*P < 0.05; **P < 0.01; ***P < 0.001.

HOMA-IR: Homeostatic model assessment of insulin resistance; HOMA- β : Homeostatic model assessment of β -cell function; BMI: Body mass index; LDL-C: Low-density lipoprotein cholesterol; ApoB: apolipoprotein B; LDLP: low density lipoprotein particles; TRLP: triglyceride rich lipoprotein particles.

Table 3

Correlations between lipids, TRL and LDL particle concentrations, subfractions and sizes with HOMA-

β						
	T2D at baseline ((n = 308), std β , P-values			No T2D at baseline (n = 4818), std β , P-values		
Variables	Univariate	Model 1	Model 2b	Univariate	Model 1	Model 2a
Non-HDL cholesterol	-0.046	-0.087	-0.014	0.086***	0.022	0.021
LDL-C	-0.041	-0.042	-0.038	0.050***	0.011	0.009
Triglycerides	0.030	-0.097	-0.084	0.183***	0.060***	0.057***
ApoB	-0.042	-0.076	-0.011	0.093***	0.026	0.025
TRLP	-0.018	-0.062	-0.030	0.091***	0.028	0.027
Very large TRLP	-0.069	-0.180**	-0.197**	0.063***	-0.034*	-0.038*
Large TRLP	0.014	-0.133*	-0.136*	0.180***	0.054***	0.049**
Medium TRLP	0.012	-0.032	0.016	0.135***	0.064***	0.067***
Small TRLP	-0.004	0.027	0.028	-0.007	0.001	0.007
Very small TRLP	0.005	-0.030	-0.005	0.012	0.016	0.015
TRL size	0.047	-0.092	-0.082	0.149***	0.040**	0.033*
LDLP	-0.030	-0.084	-0.020	0.088***	0.024	0.023
Large LDLP	-0.052	0.032	0.005	-0.112***	-0.033*	-0.030*
Medium LDLP	0.027	0.070	0.079	-0.018	0.014	0.012
Small LDLP	0.005	-0.104	-0.106	0.070***	0.024	0.022
LDL size	-0.003	0.128*	0.121*	-0.116***	-0.037*	-0.036*

β

Standardized β coefficients (Std β) are shown. Log_e transformed values are used for variables with a skewed distribution.

Model 1: sex, age, and HOMA-IR

Model 2a: model 1 + BMI, lipid-lowering medication, anti-hypertensive medication

Model 2b: model 1 + BMI, lipid-lowering medication, anti-hypertensive medication, and glucose-lowering medication

*P < 0.05; **P < 0.01; ***P < 0.001.

HOMA-IR: Homeostatic model assessment of insulin resistance; HOMA- β : Homeostatic model assessment of β -cell function; BMI: Body mass index; LDL-C: Low-density lipoprotein cholesterol; ApoB: apolipoprotein B; LDLP: low density lipoprotein particles; TRLP: triglyceride rich lipoprotein particles.

Table 4

Association between TRLP subfractions and risk of T2D in 4818 people without diabetes at baseline

HR (95% CI) per SD-increase, <i>P</i> -values							
TRLP subfractions	Total TRLP	Very large TRLP	Large TRLP	Medium TRLP	Small TRLP	Very small TRLP	TRL size
Crude	1.56 (1.36–1.80)***	1.46 (1.35–1.59)***	2.28 (1.95–2.67)***	1.50 (1.29–1.74)***	0.88 (0.79–0.98)*	1.34 (1.13–1.59)**	1.78 (1.60–1.98)***
Model 1	1.44 (1.25–1.67)***	1.46 (1.35–1.58)***	2.16 (1.84–2.54)***	1.40 (1.21–1.63)***	0.87(0.78–0.96)**	1.28 (1.09–1.51)**	1.75 (1.57–1.95)***
Model 2	1.30 (1.12–1.51)***	1.33 (1.22–1.45)***	1.71 (1.45–2.01)***	1.14 (0.99–1.32)	0.93(0.84–1.03)	1.24 (1.05–1.45)**	1.45 (1.30–1.61)***
Model 3a	1.22 (1.05–1.42)**	1.32 (1.22–1.44)***	1.62(1.37–1.90)***	1.04 (0.90–1.20)	0.93(0.84–1.03)	1.22 (1.05–1.43)*	1.42 (1.28–1.58)***
Model 3b	1.12 (0.96–1.31)	1.27 (1.16–1.38)***	1.51 (1.27–1.80)***	0.97 (0.84–1.12)	0.94(0.85–1.04)	1.18 (1.01–1.37)*	1.35 (1.21–1.51)***
Model 4a	1.26 (1.08–1.46)**	1.36 (1.25–1.47)***	1.78 (1.50–2.10)***	1.08 (0.93–1.25)	0.92(0.83–1.02)	1.23 (1.05–1.44)*	1.50 (1.35–1.67)***
Model 4b	1.12 (0.96–1.31)	1.29 (1.19–1.40)***	1.63 (1.37–1.94)***	0.98 (0.85–1.14)	0.93(0.84–1.03)	1.17 (1.01–1.36)*	1.41 (1.26–1.57)***
Model 5a	1.19 (1.03–1.38)*	1.14 (1.04–1.25)**	1.42 (1.20–1.68)***	1.09 (0.94–1.26)	0.96 (0.87–1.07)	1.19 (1.02–1.39)*	1.26 (1.12–1.41)***
Model 5b	1.12 (0.96–1.31)	1.11 (1.01–1.21)*	1.35 (1.14–1.61)**	1.04 (0.89–1.20)	0.97 (0.87–1.07)	1.16 (1.00–1.35)*	1.21 (1.07–1.36)**

HR (95% CI) per SD-increase, <i>P</i> -values
HRs (95% CIs) were derived from Cox proportional hazard models.
Model 1: sex and age
Model 2: model 1 + + high alcohol intake, BMI, lipid-lowering medication, anti-hypertensive medication, and family history of diabetes
Model 3a: model 2 + HDL-C; Model 3b: model 2 + HDL size
Model 4a: model 3a + HOMA-β; Model 4b: model 3b + HOMA-β
Model 5a: model 4a + HOMA- IR; Model 5b: model 4b + HOMA- IR
* <i>P</i> < 0.05; ** <i>P</i> < 0.01; *** <i>P</i> < 0.001.
TRLP: triglyceride rich lipoprotein particles; HOMA-IR: Homeostatic model assessment of insulin resistance; HOMA-β : Homeostatic model assessment of β-cell function; BMI: Body mass index; HDL-C: High-density lipoprotein cholesterol.

In the group with T2D at baseline, very large and large TRLP were inversely, whereas LDL size were positively associated with HOMA-β after adjustment for HOMA-IR and other variables but not in univariate models (Table 3). In the group without T2D at baseline plasma triglycerides, TRLP subfractions and size (except small and very small TRLP) were positively, whereas large LDLP and LDL size were inversely associated with HOMA-β in univariate and in multivariable models (Table 3).

Associations of HOMA-IR and HOMA-β with incident T2D

First, Cox proportional hazard regression analyses were performed for HOMA-IR and HOMA-β (Additional file 1: Table S3). HOMA-IR was associated with a higher risk of T2D in crude analyses, and after adjustment for relevant covariates. The association between HOMA-IR and incident T2D remained significant after further adjustment for HDL-C and HOMA-β or HDL size and HOMA-β [HR per 1 SD increase 5.92 (95% CI, 4.76–7.36), 5.79 (95% CI, 4.65–7.22), respectively]. Oppositely, HOMA-β was associated with a lower risk of T2D and the association between HOMA-β and incident T2D remained significant after further adjustment for HDL-C and HOMA-IR or HDL size and HOMA-IR [HR per 1 SD increase 0.21 (95% CI, 0.17–0.26), 0.20 (95% CI, 0.16–0.26), respectively].

Associations of non-HDL cholesterol, LDL-C, triglycerides, and apoB with incident T2D

In addition, Cox proportional hazard regression analyses were performed for non-HDL cholesterol, LDL-C, triglycerides, and apoB and incident T2D (Additional File1: Table S4). In crude analyses, higher concentrations of non-HDL cholesterol, LDL-C, triglycerides, and apoB were associated with an increased risk of T2D. After adjustment for relevant covariates including age and sex (models 1), high alcohol intake, BMI, family history of diabetes, anti-hypertensive and lipid-lowering medication (models 2), HDL-C and HDL size (models 3a and 3b), HOMA-β (models 4a and 4b), and HOMA-IR (models 5a and 5b), plasma triglycerides concentration was the only variable that remained significantly associated with

incident T2D. The HR per 1 SD increase in the two last models was 1.29 (95% CI, 1.13–1.47), 1.23 (95% CI, 1.07–1.42), respectively.

Associations of TRL and LDL particle subfractions and size with incident T2D

Cox proportional hazard regression analyses were performed for TRL and LDL particle subfractions and size with incident T2D (Table 5 and Table 6). Among TRL particle subfractions, higher total TRLP, very large, large, very small TRLP, and a larger TRLP size were associated with increased risk of T2D, whereas small TRLP were associated with a lower risk of T2D in crude analysis. The HR per 1 SD increase for very large TRLP in the two last models was 1.14 (95% CI, 1.04–1.25), 1.11 (95% CI, 1.01–1.21), respectively. The HR per 1 SD increase for large TRLP in the two final models was 1.42 (95% CI, 1.20–1.68) and 1.35 (95% CI, 1.14–1.61), respectively. The HR per 1 SD increase for very small TRLP in the two final models was 1.19 (95% CI, 1.02–1.39), 1.16 (95% CI, 1.00–1.35), respectively. The HR per 1 SD increase for TRL size in the two final models were 1.26 (95% CI, 1.12–1.41) and 1.21 (95% CI, 1.07–1.36), respectively.

Table 5

Association between LDL subfractions and risk of T2D in 4818 people without diabetes at baseline

HR (95% CI) per SD-increase, <i>P</i>-values					
LDL subfractions	Total LDLP	Large LDLP	Medium LDLP	Small LDLP	LDL size
Crude	1.46 (1.28–1.66)***	0.68 (0.63–0.73)***	0.84 (0.75–0.94)**	1.53 (1.25–1.87)***	0.62 (0.57–0.69)***
Model 1	1.34 (1.17–1.53)***	0.69 (0.64–0.75)***	0.82 (0.73–0.91)***	1.42 (1.18–1.71)***	0.64 (0.58–0.71)***
Model 2	1.25 (1.09–1.42)**	0.77 (0.70–0.84)***	0.90 (0.81–1.01)	1.17 (1.01–1.37)*	0.75 (0.67–0.84)***
Model 3a	1.16 (1.01–1.32)*	0.80 (0.73–0.87)***	0.89 (0.79–0.99)*	1.11 (0.96–1.28)	0.80 (0.71–0.91)***
Model 3b	1.07 (0.93–1.22)	0.83 (0.76–0.91)***	0.88 (0.78–0.98)*	1.08 (0.94–1.24)	0.86 (0.75–0.97)*
Model 4a	1.18 (1.03–1.35)*	0.77 (0.71–0.85)***	0.87 (0.78–0.98)*	1.14 (0.98–1.32)	0.78 (0.69–0.89)***
Model 4b	1.07 (0.93–1.23)	0.81 (0.74–0.89)***	0.85 (0.77–0.95)**	1.10 (0.96–1.26)	0.84 (0.74–0.96)**
Model 5a	1.11 (0.98–1.27)	0.84 (0.76–0.92)***	0.91 (0.82–1.02)	1.08 (0.94–1.23)	0.84 (0.74–0.95)**
Model 5b	1.05 (0.92–1.21)	0.86 (0.78–0.94)**	0.89 (0.80–0.99)*	1.06 (0.93–1.21)	0.87 (0.77–0.99)*
HRs (95% CIs) were derived from Cox proportional hazard models.					
Model 1: sex and age					
Model 2: model 1 + high alcohol intake, BMI, lipid-lowering medication, anti-hypertensive medication, and family history of diabetes					
Model 3a: model 2 + HDL-C; Model 3b: model 2 + HDL size					
Model 4a: model 3a + HOMA- β ; Model 4b: model 3b + HOMA- β					
Model 5a: model 4a + HOMA- IR; Model 5b: model 4b + HOMA- IR					
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.					
LDLP: low density lipoprotein particles; HOMA-IR: Homeostatic model assessment of insulin resistance; HOMA- β : Homeostatic model assessment of β -cell function; BMI: Body mass index; HDL-C: High-density lipoprotein cholesterol.					

Among LDL particle subfractions, higher concentrations of total and small LDLP, whereas lower concentration of large and medium LDLP as well as smaller LDL size were associated with increased risk of T2D in crude analyses. The association remained significant for large and medium LDLP as well as

LDL size, after adjustment for age and sex (model 1), high alcohol intake, BMI, and family anti-hypertensive and lipid-lowering medication (model 2), HDL-C or HDL size (model 3a and 3b), HOMA- β (model 4a and 4b). In the two last models adjusted for HOMA-IR (model 5a and 5b), the HR per 1 SD increase for large LDLP was 0.84 (95% CI, 0.76–0.92), 0.86 (95% CI, 0.78–0.94), respectively and the HR per 1 SD increase for LDL size was 0.84 (95% CI, 0.74–0.95), 0.87 (95% CI, 0.77–0.99), respectively. The association remained significant for medium LDLP in model 5b [HR per 1 SD increase 0.89 (95% CI, 0.80–0.99)].

Secondary analyses TRL and LDL particle subfractions and size with incident T2D in various subgroups

To find potential effect modifications, we tested for interactions by using statins (yes vs. no) and high alcohol intake (> 3 vs. ≤ 3 units per day). In crude analyses, we found significant effect modification for using statins with total TRLP ($P = 0.042$), medium TRLP ($P = 0.030$), and total LDLP ($P = 0.001$).

Consequently, secondary analyses were performed among subgroups of individuals who used statins and who did not use statins (Additional file1: S5). Higher concentrations of total TRLP and LDLP were associated with increased risk of T2D development after adjustment for age and sex (model 1), high alcohol intake, BMI, anti-hypertensive and family history of diabetes (model 2), HDL-C (model 3a), HOMA- β (model 4a), and HOMA-IR (model 5a) in non-statin users but not statin users. (Additional file1: Table S5).

In addition, an effect modification for high alcohol intake was found for large TRLP ($P = 0.042$), TRL size ($P = 0.034$), and small LDLP ($P = 0.012$). Higher levels of large TRLP, and TRL size were associated with increased risk of T2D development after adjustment for all covariates in individuals without high alcohol intake but not in subjects with high alcohol consumption (Additional file 1: Table S6).

Discussion

In the current large population-based cohort study, we investigated the association between TRL and LDL particle concentrations, determined with a novel NMR-based algorithm, with HOMA- β . In subjects without T2D at baseline, very large TRLP, large LDLP, and LDL size were inversely associated with HOMA- β , whereas large and medium TRLP and TRL size were positively associated with HOMA- β when taking account of HOMA-IR. During a median follow-up of 7.3 years, very large, large and very small TRLP and TRL size were positively associated, whereas large LDLP and LDL size were inversely associated, with incident T2D after adjustment for multiple T2DM risk factors, including BMI, HDL-C, or alternatively HDL size, HOMA- β , and HOMA-IR. In secondary analyses, higher concentrations of total TRLP and LDLP were particularly associated with a higher risk of incident T2D in non-statin users. In addition, higher levels of large TRLP, and TRL size were associated with an increased risk of T2D in participants who did not consume high amounts of alcohol.

It is well appreciated that insulin resistance plays a key role in the development of lipoprotein abnormalities featured by elevated triglycerides, higher large VLDLP and increased VLDL size, as well as

a shift from larger towards smaller LDLP and decreased LDL size [7–12]. Using a novel NMR platform-derived algorithm which captures five different TRL subfractions and three LDL subfractions such relationships with HOMA-IR were confirmed both in non-diabetic and diabetic individuals, independent of BMI, HOMA- β and other relevant covariates. In addition, a modest, but hitherto unappreciated, positive relationship of HOMA- β with TRL and an inverse relationship with LDL size was observed independent of HOMA-IR, particularly in subjects without T2D.

Regarding the associations of various TRL and LDL subfractions with incident T2D, the current results are generally consistent with prior studies, showing that NMR-measured VLDLP concentrations and size, and small LDLP concentrations were positively but large LDLP concentrations and LDL size were inversely related to incident T2D [10, 12, 23, 25, 32]. For TRL particles, we demonstrated for the first time that very large, large and very small TRLP had a relatively similar association with incident T2D. For LDL particles, higher concentration of large LDL and greater LDL size were suggested to be inversely associated, whereas higher concentrations of small LDL were positively associated with incident T2D in previous studies [9, 23, 25]. Although we found that LDL size and large LDL particles were inversely associated with risk of T2D development, we did not find a strong association between small LDL particles and incident T2D. Hence, a smaller LDL size may be associated more robustly with T2D development [9, 10, 23, 25, 33] than absolute concentrations of small LDLP. In comparison, of conventional (apo)lipoprotein and lipid measures, the plasma triglyceride concentration was the only variable that was associated with incident T2D in multivariable analysis in the whole study population at risk. Notably, since statin use is associated with an increased risk of incident T2D [34–36], we adjusted for statin use in multivariable models. Taken together, the present findings highlight the relevance of lipoprotein subfraction measurement in assessing the association of lipoprotein characteristics with the risk of T2D development. HDL particles are able to affect β -cell function [20, 37], and HDL-C makes part of established diabetic risk models [38, 39]. Recently, HDL size was found to be associated with incident T2D even independent of HDL-C [26]. In the current study, very large, large, and very small TRLP and TRL size, as well as large LDLP and LDL size, were associated with incident T2D independent of HDL-C or alternatively of HDL size.

Remarkably, the associations of TRL and LDL particle characteristics with incident T2D as documented in our study were independent of, and only modestly diminished after, adjustment for HOMA-IR despite the presently reiterated strong association of HOMA-IR with T2D development [40]. Moreover, such associations were independent of HOMA- β . In the interpretation of these results, it should be recognized that HOMA-IR is a measure of insulin resistance on glucose metabolism and hence does not necessarily represent a strong proxy of insulin resistance on free fatty acid metabolism in adipose and liver tissue [41–43]. Furthermore, HOMA- β is a static measure of insulin secretion with only has a modest relationship with dynamic tests of insulin secretory capacity by pancreatic β cells [29, 44, 45].

Evidence is mounting that cholesterol accumulation in pancreatic β -cells may give rise to β -cell dysfunction [17, 18, 46, 47]. Of further interest, VLDL and LDL particles are able to modulate β -cell function [16], possibly in part attributable to pancreatic steatosis [48]. While oxidized LDL induces β -cell

apoptosis [49], both LDL and VLDL particles may decrease β -cell proliferation by reducing cyclin B1 expression [50]. Furthermore, addition of human LDL to cultured islets impairs glucose-stimulated insulin secretion, mediated by the LDL receptor [51]. Interestingly, proprotein convertase subtilisin/kexin type 9 (PCSK9) deficiency results in increased accumulation of cholesteryl esters in pancreatic islets, coinciding with increased intracellular insulin but decreased circulating insulin [17]. In humans, circulating PCSK9 is associated with intermediate density lipoproteins [52], and high PCSK9 plasma concentrations may associate with increased risk of T2D development [18]. However, the mechanisms responsible for the association of specific TRL and LDL particle characteristics with T2D development are still unprecisely known and should await further studies.

Reasoning that associations of TRL and LDL particles characteristics with incident T2D could in part be dependent on cholesterol accumulation in pancreatic β -cells [46, 47], and that inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase could affect glucose tolerance and insulin secretion [53–55], secondary analysis were performed according to statin use. Statin users had lower levels of TC, LDL-C, non-HDL-C, apoB [56] and a slightly lower LDL particle size [57] as expected. In agreement with the possibility that exposure to higher circulating levels of lipoprotein-associated cholesterol would increase diabetes risk, higher total TRLP and LDLP concentrations were associated with incident T2D in crude and in fully adjusted analysis in non-statin users, but not in statin users. In comparison, using NMR spectroscopy (LP3 algorithm), Mackey et al. could not find a potential effect modification by use of statin on the association of NMR-measured lipoprotein particles with incident T2D [25].

Of further relevance, alcohol consumption has been included in risk scores for T2D prediction [58–60]. Although low and moderate alcohol consumption was found to be inversely associated with T2D development risk [61–64], high alcohol intake is associated with metabolic disorders including glucose, lipid and lipoprotein abnormalities, which may contribute to pancreatic toxicity [65–68]. Alcohol-induced hypertriglyceridemia is due to increased VLDL secretion, impaired lipolysis and increased free fatty acid fluxes from adipose tissue to the liver [66]. As expected, LDL-C, triglycerides, TRL subfractions, TRL size, and small LDLP were higher in subjects with high alcohol intake compared to the subjects who did not consume high amounts of alcohol [56]. Nonetheless, large TRLP and a greater TRL size were not associated with incident T2D in individuals with high alcohol consumption.

The current study has several strengths and limitations. A strength of this study is that it includes a large number of participants with a large age range, and the long-term follow-up, from the general population. Given the design of our longitudinal observation study, cause-effect relationships cannot be ascertained with certainty. Although the majority of the PREVEND participants were of north European descent, our findings are in line with previous studies which were performed in the population with different ethnicities [24, 25]. Moreover, alcohol intake was collected by participants' self-report, making that the possibility of underestimation of alcohol consumption cannot be excluded.

Conclusions

In conclusion, TRL and LDL particle characteristics are differently associated with incident T2D. Very large, large, and very small TRLP and TRL size were positively associated, whereas large LDLP and LDL size were inversely associated with incident T2D. Such associations were particularly found in non-statin users for total TRLP, and among participants who did not take high amounts of alcohol for large TRLP and TRL size.

Abbreviations

TRLP: Triglyceride-rich lipoproteins particles; **LDLP:** Low density lipoprotein particles; **T2D:** Type 2 diabetes; **PREVEND:** Prevention of Renal and Vascular End-Stage Disease; **VLDL:** Very low-density lipoproteins; **apoB:** apolipoprotein B; **NMR:** Nuclear magnetic resonance; **HOMA-IR:** Homeostasis model assessment of insulin resistance; **HOMA- β :** Homeostasis model assessment of β -cell function; **UAE:** urinary albumin excretion; **BMI:** Body mass index; **FPG:** Fasting plasma glucose; **hs-CRP:** High sensitivity C-reactive protein; **eGFR:** Estimated glomerular filtration rate; **CKD-EPI:** Chronic Kidney Disease Epidemiology Collaboration; **TC:** Total cholesterol; **HDL-C:** High density lipoprotein cholesterol; **LDL-C:** LDL-cholesterol; **PLS:** Partial Least-Squares; **PCSK9:** proprotein convertase subtilisin/kexin type 9.

Declarations

Ethics approval and consent to participate: The protocol for the PREVEND study was approved by the local ethics committee of the University Medical Center Groningen (approval number: MEC96/01/022).

Consent for publication: All the authors gave their consent to publication.

Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare no conflict of interest.

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Author Contributions: S.S. and R.P.F.D. drafted the manuscript. S.S., J.L.F.G. and R.P.F.D. contributed to the statistical analysis. M.A.C., S.J.L.B. and R.P.F.D. collected the epidemiological and clinical data. H.J.L.H., M.A.C., S.J.L.B. and R.P.F.D contributed to funding acquisition. S.J.L.B. and R.P.F.D supervised the study. S.S., J.L.F.G., H.J.L.H., M.A.C., S.J.L.B. and R.P.F.D revised the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

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