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HDL Particle Subspecies and Their Association With Incident Type 2 Diabetes: The PREVEND Study

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Abbreviations: BMI, body mass index; CEC, cellular cholesterol efflux; CETP, cholesterol ester transfer protein; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; H1P-H7P, HDL subspecies by particle diameter ("small" HDL includes H1-H2, "medium" H3-H4, and "large" H5-H7); HDL-C, high-density lipoprotein cholesterol; HDL-P, total HDL particle concentration; HOMA-IR, homeostatic model assessment of insulin resistance; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NMR, nuclear magnetic resonance; PLTP, phospholipid transfer protein; PREVEND, Prevention of Renal and Vascular ENd-stage Disease; T2D, type 2 diabetes.

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Abstract

Context: High-density lipoproteins (HDL) may be protective against type 2 diabetes (T2D) development, but HDL particles vary in size and function, which could lead to differential associations with incident T2D. A newly developed nuclear magnetic resonance (NMR)-derived algorithm provides concentrations for 7 HDL subspecies.

Objective: We aimed to investigate the association of HDL particle subspecies with incident T2D in the general population.

Methods: Among 4828 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study without T2D at baseline, HDL subspecies with increasing size from H1P to H7P were measured by NMR (LP4 algorithm of the Vantera NMR platform).

Results: A total of 265 individuals developed T2D (median follow-up of 7.3 years). In Cox regression models, HDL size and H4P (hazard ratio [HR] per 1 SD increase 0.83 [95% CI, 0.69-0.99] and 0.85 [95% CI, 0.75-0.95], respectively) were inversely associated with incident T2D, after adjustment for relevant covariates. In contrast, levels of H2P were positively associated with incident T2D (HR 1.15 [95% CI, 1.01-1.32]). In secondary analyses, associations with large HDL particles and H6P were modified by body mass index (BMI) in such a way that they were particularly associated with a lower risk of incident T2D, in subjects with BMI < 30 kg/m^2.

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Conclusion: Greater HDL size and lower levels of H4P were associated with a lower risk, whereas higher levels of H2P were associated with a higher risk of developing T2D. In addition, large HDL particles and H6P were inversely associated with T2D in nonobese subjects.

Key Words: HDL cholesterol, HDL particles, HDL subspecies, type 2 diabetes mellitus, obesity

The prevalence of type 2 diabetes (T2D) as one of the most common metabolic disorders is rapidly increasing (1). A better understanding of the pathophysiology of the condition will help to prevent or delay the onset of T2D by various interventions (2). It is established that high-density lipoprotein cholesterol (HDL-C) is inversely associated with risk of developing T2D as described previously in different ethnic groups (3-6). However, HDL particles are heterogeneous and vary in composition, size, and function, which may lead to differential associations with incident T2D (7, 8). Larger and smaller HDL particles have been reported to relate differently to lipid transport, as well as to anti-inflammatory and antioxidative functions (9-12). In previous size-based HDL particle analyses, lower levels of large HDL particles and higher levels of smaller HDL particles were more likely to be associated with insulin resistance and the development of T2D; however, associations of medium and small HDL particles with incident T2D have been inconsistently reported (13-19).

A newly developed nuclear magnetic resonance (NMR)-derived algorithm called LP4 provides concentrations for 7 HDL subspecies and categorizes HDL particles into small, medium, and large HDL more specifically (20-22). This allows for additional HDL subspecies interrogation which may help to improve prediction of new-onset T2D compared with the use of HDL-C and other measures of HDL particle characteristics.

Of further relevance, HDL composition and function is likely to be disturbed in the context of obesity and the metabolic syndrome (23, 24), making it relevant to discern whether the association of HDL particle parameters with incident T2D varies according to obesity and insulin resistance. It is also noteworthy that HDL-C and larger sized HDL particles are increased in women (25, 26), coinciding with lower diabetes incidence (27).

The current study was initiated to explore the associations of HDL variables, namely, HDL-C, HDL particle concentration (HDL-P), large, medium, and small HDL particles, HDL size, and 7 HDL subspecies with incident T2D in the general population. Second, we aimed to examine the influence of obesity, insulin resistance, and sex on the association between these HDL parameters and newly developed T2D.

Methods

Study Design and Participants

This study was conducted within the framework of the Prevention of Renal and Vascular ENd-stage Disease (PREVEND) study, a prospective Dutch cohort study in a general population among inhabitants, aged from 28 to 75 years, of the city Groningen, the Netherlands. The details of the study design and recruitment have been described before (28). Briefly, after exclusion of individuals using insulin and pregnant women, 7768 subjects with urinary albumin concentration ≥10 mg/L and 2592 individuals with urinary albumin concentration <10 mg/L were invited to participate in the study. The PREVEND study initially included 8592 subjects who completed the study screening program (1997-1998). The second screening was the starting point of the current study (2001-2003) and included 6892 subjects. 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 preexisting diabetes and 4828 subjects without diabetes at baseline of the present study.

The PREVEND study was approved by the local medical ethics committee, University Medical Center Groningen (approval number: MEC96/01/022) and was performed according to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

Clinical and Laboratory Measurement

Participants attended 2 outpatient visits during which baseline data were collected on demographics, lifestyle parameters, anthropometric measurements, parental history of T2D, medical history, and medication use. Information on medication use was combined with information from a pharmacy-dispensing registry, which had complete information on the drug usage of >95% of subjects in the PREVEND study (29). Smoking status as well as alcohol use, calculation of body mass index (BMI), and measurement of blood pressure, have been described previously (30). Hypertension was defined by self-reported physician diagnosis, use of antihypertensive medication, or blood pressure ≥140/90 mmHg. Homeostatic model assessment of insulin

resistance (HOMA-IR) was calculated as fasting plasma insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5 (31).

Fasting venous plasma and serum samples were taken from participants after an overnight fast. 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). High-sensitivity C-reactive protein (hs-CRP) was assayed by nephelometry (Dade Behring Diagnostic, Marburg, Germany). Serum creatinine and cystatin C measurement have been described previously (32). Urinary albumin was measured by nephelometry (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 spectroscopy at LabCorp (Morrisville, NC, formerly LipoScience) using an optimized version of the NMR LipoProfile test (LP4 algorithm) on plasma EDTA specimens (20, 22, 33). Total cholesterol, triglycerides, and HDL cholesterol (HDL-C) were measured using Vantera Clinical NMR Analyzer platform using Partial Least-Squares (PLS) regression models. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Small, medium, and large HDL particles and HDL subspecies (H1P to H7P; see below) were quantified using the amplitudes of their spectroscopically distinct lipid methyl group NMR signals (34). Total HDL particles (HDL-P) was calculated by the sums of the concentrations of small, medium, and large HDL particles. Mean HDL size was calculated using the weighted averages derived from the sum of the diameters of small, medium, and large HDL particles multiplied by its relative mass percentage. Estimated ranges of particle diameter for the subclasses and subspecies were as follows: small HDL, 7.4 to 8.0 nm; medium HDL, 8.1 to 9.5 nm; large HDL, 9.6 to 13 nm; H1P, 7.4 nm; H2P, 7.8 nm; H3P, 8.7 nm; H4P, 9.5 nm; H5P, 10.3 nm; H6P, 10.8 nm; and H7P, 12.0 nm. Small HDL comprises H1 and H2, medium HDL, H3 and H4, and large HDL, H5 to H7.

Outcome Ascertainment

Follow-up time was defined as the period between the baseline measurement (second screening) and the date of ascertainment of T2D. Follow-up time was censored at 7.3 years. In case a person moved to an unknown destination, census date was 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 with the use of the statistical packages IBM SPSS (version 24.0.1; SPSS, Chicago, IL, USA) and STATA/SE (version 14; StataCorp, College Station, TX, USA). Continuous variables were compared by using one-way ANOVA or a Kruskal Wallis test for skewed variables with subsequent Bonferroni correction, and a X^2 tests for categorical variables to test for differences across participants with preexisting diabetes, and with and without incident T2D. Data were expressed as mean ± standard deviation (SD), median with interquartile range (IQR) or as numbers (percentages). Univariate as well as multivariate Pearson correlation coefficients were estimated to assess the cross-sectional correlations of HDL variables (HDL-C, HDL-P, large, medium, and small HDL particles, and HDL size) and HDL subspecies (H1-H7) with participants' characteristics and laboratory parameters. Variables with a skewed distribution were log transformed. Crude and multivariable Cox proportional hazards regression analysis was 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 transformed for variables which were not normally distributed). The following 5 cumulative models were used for adjustment: age and sex (models 2); plus family history of diabetes (models 3); plus other established T2D risk factors including smoking status, alcohol consumption, BMI, hypertension, hs-CRP, and use of lipid-lowering medication (models 4); plus FPG (models 5a) or HOMA-IR (models 5b); plus FPG, LDL-C, and triglycerides (models 6a) or plus HOMA-IR, LDL-C, and triglycerides (models 6b). We tested interactions to assess statistical evidence of for effect modification by BMI, sex, and HOMA-IR in crude analyses. For predictors of which the interaction test was significant, we performed subgroup analyses in HRs across categories of prespecified subject characteristics, including BMI (<30 kg/m^2 vs ≥30 kg/m^2), sex (men vs women), and HOMA-IR (continuous and < median vs ≥ median value) in all 5 adjusted models. Two-sided P values < 0.05 were considered statistically significant.

Results

Baseline Characteristics

Baseline characteristics of 308 subjects with preexisting diabetes and 4828 individuals without T2D at study start

are shown in Table 1. After a median follow-up period of 7.3 years, 265 individuals developed T2D. People with preexisting diabetes and those who developed T2D were more likely to be men and to be older, and they were more likely to have a family history of diabetes when compared with people who did not develop T2D during follow-up. Individuals with preexisting diabetes consumed less alcohol compared with people who developed and who did not develop T2D. People with preexisting diabetes and those who developed T2D had a higher BMI, a larger waist circumference, higher systolic and diastolic blood pressure, and higher FPG, fasting insulin, HOMA-IR, and hs-CRP. In subjects with preexisting diabetes and who developed T2D, urinary albumin excretion was higher, and eGFR was lower. They used antihypertensive medications and lipid-lowering drugs more frequently. Total cholesterol was higher in those who developed T2D compared with those with preexisting diabetes and who did not develop T2D. Triglycerides and small HDL particles were higher, whereas HDL-C, HDL-P, large and medium HDL particles, and HDL size were lower in people with preexisting diabetes and who developed T2D. Among HDL subspecies, H1P, H3P, H4P, H6P, and H7P levels were lower in subjects with preexisting diabetes and who developed T2D, but H2P levels were higher. No significant difference among the groups were found in their LDL-C and H5P levels.

Cross-Sectional Associations

As shown in Table S1 (35), we performed multivariable analyses to show the associations between HDL variables (HDL-C, HDL-P, large, medium, and small HDL particles, and HDL size) and variables of interest for which a significant association was found in the univariate models (data not shown). Female sex was positively associated with HDL-C, HDL-P, large and medium HDL particles, and HDL size, and inversely associated with small HDL particles. Having a positive family history of diabetes was inversely associated with large HDL particles and HDL size. BMI, HOMA-IR, triglycerides, and LDL-C were inversely associated with all HDL variables, except with small HDL particles.

In further cross-sectional multivariable regression analyses (Table S2 (35)), we assessed the association between each of the 7 HDL subspecies (H1P to H7P) and variables of interest with those variables with a significant association in univariate analyses was found (data not shown). Female sex was inversely associated with small subspecies (H1P and H2P) but positively associated with several medium-sized and large subspecies (H3P, H4P, H6P, H7P). Family history of diabetes was positively associated with H2P, but inversely with H6P. BMI, HOMA-IR, and triglycerides were positively associated with H2P, and inversely with H6P and H7P.

Furthermore, HDL-C was correlated positively with medium and large HDL particles, and inversely with small HDL particles (Table S3 (35)). Large and medium HDL particles were positively correlated whereas negative correlations between small HDL particles with medium and large HDL particles were found (Table S3 (35)). In addition, HDL-C was negatively correlated with H1P, H2P, and H5P, and positively with H3P, H4P, H6P, and H7P (Table S3 (35)).

Association of HDL-C, HDL-P, Large, Medium, and Small HDL, and HDL Size With Incident T2D

During a median follow-up of 7.3 (6.1-7.7) years, 265 of the 4828 subjects without diabetes at baseline developed T2D. Cox proportional hazard regression analyses were first performed for HDL-C, HDL-P, large, medium, and small HDL particles, and HDL size (Table 2). In the crude model, higher levels of HDL-C, HDL-P, large, medium HDL particles, and HDL size were associated with a lower risk of T2D, whereas higher levels of small HDL particles were associated with an increased risk of T2D. After cumulative adjustment for age and sex (models 2), family history of diabetes (models 3), smoking, alcohol use, BMI, hs-CRP, hypertension, and use of lipid-lowering medication (models 4), and FPG (models 5a), the association remained significant for HDL-C, HDL size, small, medium, and large HDL particles. After adjustment for HOMA-IR instead of FPG (models 5b) and in 2 last models, in which we additionally adjusted for LDL-C and triglycerides (models 6a and 6b), HDL size remained inversely associated with incident T2D (HR per 1 SD increase 0.76 [95% CI, 0.64-0.91], 0.77 [95% CI, 0.63-0.95], and 0.83 [95% CI, 0.69-0.99], respectively).

Association of HDL Subspecies With Incident T2D

In addition, Cox proportional hazard regression analyses were performed for the 7 HDL subspecies and incident T2D (Table 3). In the crude model, higher concentrations of H1P, H3P, H4P, H6P, and H7P were associated with lower risk of T2D (Table 3), whereas higher concentrations of H2P were associated with an increased risk of T2D. After adjustment for relevant covariates, including age and sex (models 2), family history of diabetes (models 3), smoking, alcohol use, BMI, hs-CRP, hypertension, use of lipid-lowering medication (models 4), and FPG (models 5a), the association remained significant for H2P, H4P, H6P, and H7P. After adjustment

Table 1. Baseline Characteristics of 308 Subjects With Preexisting Diabetes at Baseline, 265 Subjects Who Developed T2D, and 4563 Subjects Who Did Not Develop T2D in the Span of 7.3 Years

Variables	Diabetes at baseline	Incident diabetes		*P* value
		Yes	No	
Participants, n	308	265	4563	
General characteristics				
Female, %	43.8	37.7	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	27.9	26.8	0.641
Alcohol use, %	59.4	73.1	76.9	<0.001[a c]
Family history of diabetes %	31.5	32.8	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	0.253
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	90.7 ± 12.2	<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]
Diastolic blood pressure, mmHg	75.3 ± 9.0	77.3 ± 9.0	72.9 ± 8.9	<0.001[a b c]
Hypertension, %	63.6	55.8	25.8	<0.001[a b c]
Antihypertensive medication, %	44.8	35.1	14.1	<0.001[a b c]
Lipid-lowering medication, %	26.3	19.0	7.3	<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 (2.3–5.3)	1.6 (1.1–2.4)	<0.001[a b]
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)	8.3 (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]
LDL-C, mg/dL	112.7 ± 32.6	118.2 ± 30.0	113.9 ± 29.1	0.045
HDL-C, mg/dL	44.6 ± 9.9	45.9 ± 9.9	52.0 ± 12.2	<0.001[a b]
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]
HDL-P, µmol/L	20.1 ± 3.0	20.7 ± 2.8	21.2 ± 2.7	0.004[a b c]
Large HDL, µmol/L	0.9 (0.7–1.6)	1.0 (0.6–1.5)	1.5 (0.9–2.5)	<0.001[a b]
Medium HDL, µmol/L	3.9 (2.8–5.4)	4.3 (3.0–5.7)	5.1 (3.7–6.5)	<0.001[a b]
Small HDL, µmol/L	14.7(12.8–16.4)	14.9 ± 2.7	14.1 ± 2.9	<0.001[a b]
HDL size, nm	8.7 ± 0.3	8.7 ± 0.4	9.00 ± 0.4	<0.001[a b]
HDL Subspecies				
H1P, µmol/L	3.1 (1.6–4.2)	3.1 (1.9–4.3)	3.5 (2.3–4.7)	<0.001[a b]
H2P, µmol/L	11.6 (10.0–13.1)	11.7 (10.2–13.1)	10.5 (9.9–12.1)	<0.001[a b]
H3P, µmol/L	2.6 (1.4–3.8)	2.9 (1.7–4.2)	3.2 (2.1–4.4)	<0.001[a b]
H4P, µmol/L	1.3 (0.8–1.9)	1.3 (0.7–2.0)	1.7 (1.2–2.5)	<0.001[a b]
H5P, µmol/L	0.3 (0.1–0.7)	0.3 (0.1–0.6)	0.3 (0.1–0.6)	0.280
H6P, µmol/L	0.4 (0.2–0.7)	0.4 (0.2–0.7)	0.6 (0.3–1.4)	<0.001[a b]
H7P, µmol/L	0.2 (0.1–0.4)	0.2 (0.1–0.4)	0.3 (0.1–0.6)	<0.001[a b]

Data are the mean ± SD, median (interquartile range) unless otherwise indicated. Significance was tested by one-way ANOVA tests and Kruskal Wallis tests where appropriate.

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; H1P-H7P, high-density lipoprotein 1-7 particles (from smallest to largest); HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particles; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; T2D, type 2 diabetes.

[a]Different between the group with T2D at baseline and the group without T2D at follow-up. [b]Different between the group with incident T2D and the group without T2D at follow-up. [c]Different between the group with T2D at baseline and the group with T2D at baseline and the group with incident T2D at follow-up at *P* value < 0.05 (by Bonferroni correction).

Table 2. Association Between HDL Variables and Risk of T2D in 4828 Individuals Without Diabetes at Baseline

HR (95% CI) per SD increase, *P* values

HDL Variables	HDL-C	HDL-P	Large HDL	Medium HDL	Small HDL	HDL size
Model 1	**0.58 (0.51–0.66)*** **	**0.81 (0.71–0.92)** **	**0.64 (0.57–0.71)*** **	**0.73 (0.66–0.81)*** **	**1.31 (1.16–1.48)*** **	**0.50 (0.43–0.58)*** **
Model 2	**0.59 (0.52–0.68)*** **	**0.86 (0.76–0.98)***	**0.63 (0.56–0.70)*** **	**0.78 (0.70–0.88)*** **	**1.23 (1.09–1.40)** **	**0.48 (0.41–0.56)*** **
Model 3	**0.60 (0.52–0.69)*** **	**0.86 (0.76–0.98)***	**0.64 (0.57–0.72)*** **	**0.79 (0.70–0.88)*** **	**1.22 (1.07–1.38)** **	**0.48 (0.41–0.57)*** **
Model 4	**0.76 (0.65–0.88)*** **	0.97 (0.85–1.10)	**0.77 (0.67–0.87)*** **	**0.90 (0.80–1.01)***	**1.14 (1.00–1.30)***	**0.62 (0.52–0.74)*** **
Model 5a	**0.84 (0.69–0.94)** **	1.01 (0.89–1.15)	**0.83 (0.73–0.95)** **	**0.87 (0.77–0.98)***	**1.18 (1.04–1.34)***	**0.67 (0.56–0.80)*** **
Model 5b	0.87 (0.75–1.02)	1.00 (0.88–1.14)	0.90 (0.78–1.03)	0.93 (0.83–1.05)	1.08 (0.95–1.24)	**0.76 (0.64–0.91)** **
Model 6a	0.89 (0.76–1.05)	1.01 (0.89–1.15)	0.92 (0.80–1.07)	0.94 (0.83–1.06)	1.07 (0.94–1.23)	**0.77 (0.63–0.95)***
Model 6b	0.94 (0.79–1.10)	1.01 (0.89–1.15)	0.96 (0.82–1.11)	0.99 (0.87–1.12)	1.03 (0.90–1.18)	**0.83 (0.69–0.99)***

Hazard ratios (HRs) with 95% CIs were derived from Cox proportional hazard models. Significant associations are shown in bold.

Model 1: crude model; Model 2: model 1 + age, sex; Model 3: model 2 + family history of diabetes; Model 4: model 3 + smoking, alcohol consumption, body mass index, hypertension, high-sensitivity C-reactive protein, and use of lipid-lowering medication; Model 5a: model 4 + fasting plasma glucose; Model 5b: model 4 + homeostatic model assessment of insulin resistance; Model 6a: model 5a + low-density lipoprotein cholesterol (LDL-C), triglycerides; Model 6b: model 5b + LDL-C, triglycerides.

Abbreviations: HDL, high-density lipoprotein; T2D, type 2 diabetes.

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

Table 3. Association Between HDL Subspecies and Risk of T2D in 4828 Individuals Without Diabetes at Baseline

HR (95% CI) per SD increase, *P* values

HDL Subspecies	H1P	H2P	H3P	H4P	H5P	H6P	H7P
Model 1	**0.81(0.73–0.90)*** **	**1.56 (1.40–1.74)*** **	**0.83 (0.75–0.92)** **	**0.66 (0.60–0.72)*** **	1.03 (0.91–1.16)	**0.64 (0.57–0.72)*** **	**0.59 (0.52–0.67)*** **
Model 2	**0.79 (0.71–0.87)*** **	**1.50 (1.34–1.68)*** **	0.89 (0.80–1.00)	**0.68 (0.62–0.75)*** **	1.01 (0.89–1.14)	**0.64 (0.56–0.72)*** **	**0.60 (0.53–0.69)*** **
Model 3	**0.79 (0.71–0.87)*** **	**1.49 (1.32–1.67)*** **	0.89 (0.80–1.00)	**0.69 (0.63–0.76)*** **	1.01 (0.90–1.14)	**0.65 (0.57–0.74)*** **	**0.61 (0.53–0.70)*** **
Model 4	**0.87 (0.78–0.96)** **	**1.33 (1.18–1.50)*** **	0.99 (0.88–1.11)	**0.74 (0.67–0.82)*** **	1.03 (0.91–1.17)	**0.77 (0.67–0.88)*** **	**0.72 (0.62–0.83)*** **
Model 5a	0.98 (0.88–1.09)	**1.28 (1.13–1.45)*** **	0.92 (0.82–1.03)	**0.80 (0.72–0.89)*** **	0.97 (0.86–1.10)	**0.85 (0.74–0.99)***	**0.77 (0.67–0.89)*** **
Model 5b	0.91 (0.81–1.01)	**1.22 (1.08–1.38)** **	0.99 (0.88–1.11)	**0.81 (0.73–0.90)*** **	1.07 (0.93–1.21)	0.87 (0.75–1.01)	**0.83 (0.72–0.95)** **
Model 6a	1.02 (0.92–1.14)	**1.15 (1.01–1.32)***	0.96 (0.86–1.08)	**0.87 (0.77–0.98)***	1.02 (0.89–1.16)	0.91 (0.78–1.05)	0.87 (0.74–1.01)
Model 6b	0.92 (0.83–1.03)	**1.15 (1.01–1.32)***	1.01 (0.90–1.14)	**0.85 (0.75–0.95)** **	1.08 (0.94–1.23)	0.90 (0.77–1.04)	0.89 (0.76–1.04)

Hazard ratios (HRs) with 95% CIs were derived from Cox proportional hazard models. Significant associations are shown in bold.

Model 1: crude model; Model 2: model 1 + age, sex; Model 3: model 2 + family history of diabetes; Model 4: model 3 + smoking, alcohol consumption, body mass index, hypertension, high-sensitivity C-reactive protein, and use of lipid-lowering medication; Model 5a: model 4 + fasting plasma glucose; Model 5b: model 4 + homeostatic model assessment of insulin resistance; Model 6a: model 5a + low-density lipoprotein cholesterol (LDL-C), triglycerides; Model 6b: model 5b + LDL-C, triglycerides.

Abbreviations: H1P-H7P, high-density lipoprotein 1-7 particles (from smallest to largest); HDL, high-density lipoprotein; T2D, type 2 diabetes.

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

for HOMA-IR instead of FPG (models 5b) and in 2 last models, in which we additionally adjusted for LDL-C and triglycerides (models 6a and 6b), H4P remained inversely associated with incident T2D (HR per 1 SD increase 0.81 [95% CI, 0.73-0.90], 0.87 [95% CI, 0.77-0.98] and 0.85 [95% CI, 0.75-0.95], respectively), while H2P remained positively associated with incident T2D (HR per 1 SD increase 1.22 [95% CI, 1.08-1.38], 1.15 [95% CI, 1.01-1.32] and 1.15 [95% CI, 1.01-1.32], respectively).

Sensitivity Analyses on the Association Between Large, Medium and Small HDL Particles and HDL Subspecies With Incident T2D

For sensitivity analyses, we used the proportions of large, medium, and small HDL particles as a fraction of total HDL-P, as well as the proportions of H1P to H7P as a fraction of total HDL-P. The proportion of large HDL with a lower risk of incident T2D remained significant after adjustment for FPG or HOMA-IR (Table S4, models 5a and 5b) (35). H2P was associated with an increased risk of T2D, whereas H4P was inversely associated with incident T2D (Table S5, model 6b (35)).

Influence of HDL-C on the Association of Large, Medium, and Small HDL Particles and HDL Subspecies With Incident T2D

To determine potential confounding in the regression models, analyses were performed with HDL-C as an additional independent variable and with HDL-C and HDL subspecies jointly in the same model. The inverse association of large HDL with incident T2D remained significant after adjustment for HDL-C, whereas the positive association between small HDL particles and incident T2D remained significant when taking account of HDL-C, as well as of large and medium HDL particles (Table S6 (35)). Also, in analyses adjusted for age, sex, and HDL-C, higher concentrations of H2P were associated with an increased risk of T2D, whereas H1P, H4P, H6P, and H7P were inversely associated with incident T2D (Table S7 (35)). In analyses jointly adjusted for the various HDL subspecies, H1P and H4P were inversely and H2P was positively associated with increased T2D risk.

Secondary Analyses of HDL Variables and Subspecies With Incident T2D in Various Subgroups

To find potential effect modifications, we tested for interactions by BMI (dichotomized as <30 kg/m^2 and ≥30 kg/m^2) and sex with HDL-C, HDL-P, large, medium, and small HDL particles, HDL size, and the 7 HDL subspecies. In crude analyses, we found significant effect modification for BMI with large HDL ($P = 0.041$) and H6P ($P = 0.008$). Consequently, we performed secondary analyses within subgroups of individuals with BMI < 30 kg/m^2 and BMI ≥ 30 kg/m^2. Higher levels of large HDL and H6P were associated with a lower risk of incident T2D in subjects with BMI < 30 kg/m^2 after adjustment for relevant covariates, whereas these associations were not significant in subjects with BMI ≥ 30 kg/m^2 (Table 4).

In addition, effect modification for sex was found for large HDL ($P = 0.029$), small HDL ($P = 0.028$), H5P ($P = 0.015$), and H6P ($P = 0.001$). Higher levels of large HDL particles and H6P were associated with a lower risk of T2D development in women, but not in men after adjustment for relevant covariates (Table 5). There was no significant (adjusted) association between small HDL particles and H5P with incident T2D in men and in women separately.

Of further note, effect modification for HOMA-IR as a continuous variable was observed for H3P ($P = 0.005$), H5P ($P = 0.004$), and H6P ($P = 0.001$). In dichotomized and unadjusted analysis, the inverse association between H6P and incident T2D was stronger in subjects with HOMA-IR < the median value of the whole group of 1.68 (mU/L^2)/22.5 (HR 0.62; 95% CI, 0.44-0.87 per 1 SD increase; $P = 0.006$) compared to subjects with HOMA-IR ≥ 1.68 (mU/L^2)/22.5 (HR 0.80; 95% CI, 0.70-0.92 per 1 SD increase; $P = 0.001$) (data not shown). However, after adjustment for all covariates (model 6a in previous Tables), the association between H6P and incident T2D did not reach significance in each of the HOMA-IR subgroups (HR 0.76; 95% CI, 0.51-0.1.13 per 1 SD increase; $P = 0.17$ in the low HOMA-IR subgroup vs HR 0.91; 95% CI, 0.78-1.05 per 1 SD increase; $P = 0.19$ in the high HOMA-IR subgroup).

Discussion

In this large population based-cohort study, we discerned the association of HDL size and various HDL subclasses (small, medium, and large HDL particles) and subspecies (H1P-H7P) determined with a novel NMR-based algorithm with incident T2D in the general population. We found that larger HDL size and higher H4P concentrations were inversely associated with T2D development, whereas H2P levels were positively associated with T2D development after multiple adjustments. In secondary analyses, large HDL particles and H6P were also inversely associated with T2D in fully adjusted analyses, in subjects with BMI < 30 kg/m^2. In obese subjects, in contrast, such independent associations were not observed. Furthermore,

Table 4. Association Between HDL Variables and Subspecies at Baseline With Risk of T2D in Low and High BMI Subgroups or Parameters With Significant Interaction With BMI

	Large HDL	H6P
BMI < 30 kg/m^2 (n = 4007)		
Events	152	152
Model 1	**0.64 (0.55–0.73)*****	**0.61 (0.52–0.71)*****
Model 2	**0.64 (0.55–0.75)*****	**0.61 (0.52–0.73)*****
Model 3	**0.66 (0.56–0.77)*****	**0.64 (0.54–0.76)*****
Model 4	**0.71 (0.60–0.84)*****	**0.70 (0.59–0.83)*****
Model 5a	**0.76 (0.64–0.90)****	**0.78 (0.65–0.93)****
Model 5b	0.85 (0.71–1.01)	**0.81 (0.68–0.97)***
Model 6a	**0.82 (0.68–0.98)***	**0.83 (0.69–0.99)***
Model 6b	0.93 (0.77–1.12)	0.85 (0.71–1.02)
BMI ≥ 30 kg/m^2 kg/m^2(n = 821)		
Events	113	113
Model 1	**0.81 (0.67–0.96)***	0.86 (0.70–1.05)
Model 2	**0.80 (0.66–0.97)***	0.86 (0.70–1.07)
Model 3	**0.81 (0.66–0.98)***	0.86 (0.70–1.08)
Model 4	**0.81 (0.66–0.99)***	0.86 (0.69–1.07)
Model 5a	0.88 (0.71–1.08)	1.01 (0.79–1.28)
Model 5b	0.88 (0.71–1.10)	0.93 (0.74–1.17)
Model 6a	0.92 (0.73–1.16)	1.06 (0.83–1.36)
Model 6b	0.92 (0.73–1.16)	0.96 (0.76–1.21)

Hazard ratios (HRs) with 95% CIs were derived from Cox proportional hazard models. Significant associations are shown in bold.

Model 1: crude model; Model 2: model 1 + age, sex; Model 3: model 2 + family history of diabetes; Model 4: model 3 + smoking, alcohol consumption, body mass index, hypertension, high-sensitivity C-reactive protein, and use of lipid-lowering medication; Model 5a: model 4 + fasting plasma glucose; Model 5b: model 4 + homeostatic model assessment of insulin resistance; Model 6a: model 5a + low-density lipoprotein cholesterol (LDL-C), triglycerides; Model 6b: model 5b + LDL-C, triglycerides.

Abbreviations: BMI, body mass index; H6P, high-density lipoprotein 6 particles (10.8 nm); HDL, high-density lipoprotein; T2D, type 2 diabetes.

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

higher concentrations of large HDL and H6P were associated with incident T2D in women.

The inverse association of HDL-C with T2D development as shown in earlier epidemiological studies has been translated into a potential protective influence of HDL-C against incident T2D (4-6). Consequently HDL-C has been included in risk scores of T2D prediction drawn from the Framingham Offspring Study Diabetes Mellitus and the Diabetes Prediction Model risk scores (3, 5). HDL does not represent a single lipoprotein fraction (commonly assessed by the HDL-C concentration) but includes a diverse group of particles which may differ in their alleged antidiabetic roles (9-12, 36). In the current study, HDL-C was positively associated with large and medium HDL, as well as with HP3, HP4, HP6, and HP7, and inversely with small HDL as well as with HP1, HP2, and HP5. A potentially important novel observation of our study is the inverse association of large HDL and the positive association small HDL with incident T2D remained after adjustment for age, sex, and HDL-C. This suggest that such associations of NMR-assayed HDL subfractions may be clinically meaningful beyond measurement of HDL-C per se.

The association between different HDL particle subclasses (small, medium, and large) and HDL size with incident T2D has been studied recently, including application of NMR methodology. Higher concentrations of large HDL and greater HDL size were suggested to be inversely associated with incident T2D (8, 17-19). However, the association between medium and small HDL particles and incident T2D was evaluated only in a limited number of studies (16-18). These previous studies were conducted in different populations with varying ethnicities, and the HDL variables studied were not uniformly adjusted for covariates (16-19). Interestingly, the current study expanded the investigation of HDL with incident T2D by assessing its association with 7 different HDL subspecies and demonstrated for the first time the positive and inverse associations of H2P and H4P, respectively, with incident T2D in adjusted analyses. Furthermore, the association between the larger subspecies H6P and H7P and incident T2D were independent of metabolic factors but not of HOMA-IR, triglycerides, and LDL-C. In comparison, we recently documented an inverse association of H6P with development of posttransplantation diabetes in renal transplant recipients (22).

A salient feature of HDL subclasses and subspecies distribution and their associations with T2D could be explained by their relationships with BMI and insulin resistance as confirmed in the current study. Insulin resistance is appreciated to be associated with fewer large HDL particles and a decrease in HDL particle size (15, 16, 37, 38). Of further interest, the correlation between phospholipid content of HDL subfractions separated by gel filtration chromatography and HDL subspecies as quantified by NMR spectroscopy was reported recently (37, 39). Fractions with low phospholipid content appeared very similar to H7P and H6P, respectively, as assessed by NMR, while the fraction with high phospholipid content appeared very similar to H2P as assessed by NMR (37). Fractions with a low phospholipid content were inversely correlated with HOMA-IR (37). Moreover, HDL remodeling consequent to insulin resistance and T2D results in HDL particles with fewer cholesteryl esters (40, 41). Taken together, these findings make it necessary to adjust for adiposity and/or insulin resistance when evaluating the association of HDL subfractions and subspecies with newly developed T2D.

Table 5. Association Between HDL Variables and Subspecies and Risk of T2D in 2390 Men and 2438 Women for Parameters With Significant Interaction With Sex

	Large HDL	Small HDL	H5P	H6P
Men				
Events	165	165	165	165
Model 1	**0.73 (0.62–0.85)*****	1.09 (0.93–1.30)	0.89 (0.75–1.05)	**0.80 (0.67–0.94)****
Model 2	**0.69 (0.59–0.80)*****	1.14 (0.96–1.35)	0.89 (0.75–1.05)	**0.75 (0.64–0.89)****
Model 3	**0.70 (0.60–0.82)*****	1.12 (0.953–1.33)	0.90 (0.77–1.06)	**0.77 (0.65–0.91)****
Model 4	0.87 (0.74–1.06)	1.05 (0.88–1.24)	0.98 (0.83–1.17)	0.94 (0.78–1.13)
Model 5a	0.97 (0.81–1.15)	1.17 (0.99–1.38)	0.93 (0.79–1.10)	1.01 (0.84–1.23)
Model 5b	0.98 (0.81–1.17)	1.03 (0.87–1.23)	1.01 (0.85–1.20)	1.02 (0.84–1.23)
Model 6a	1.09 (0.90–1.32)	1.06 (0.86–1.31)	0.98 (0.82–1.17)	1.05 (0.86–1.28)
Model 6b	1.04 (0.85–1.27)	0.99 (0.83–1.18)	1.03 (0.86–1.23)	1.05 (0.86–1.28)
Women				
Events	100	100	100	100
Model 1	**0.56 (0.47–0.67)*****	**1.45 (1.21–1.75)*****	**1.21 (1.01–1.45)***	**0.53 (0.44–0.63)*****
Model 2	**0.56 (0.47–0.67)*****	**1.33 (1. 1–1.61)****	1.17 (0.97–1.41)	**0.52 (0.43–0.63)*****
Model 3	**0.57 (0.47–0.67)*****	**1.31 (1.09–1.59)****	1.16 (0.96–1.39)	**0.53 (0.44–0.64)*****
Model 4	**0.66 (0.54–0.82)*****	1.19 (0.96–1.46)	1.08 (0.89–1.31)	**0.63 (0.51–0.79)*****
Model 5a	**0.72 (0.58–0.89)****	1.17 (0.95–1.43)	1.04 (0.85–1.28)	**0.75 (0.60–0.94)***
Model 5b	0.84 (0.67–1.05)	1.09 (0.88–1.35)	1.10 (0.89–1.35)	**0.75 (0.60–0.94)***
Model 6a	**0.77 (0.61–0.97)***	1.10 (0.93–1.31)	1.07 (0.87–1.31)	**0.77 (0.61–0.97)***
Model 6b	0.88 (0.70–1.11)	1.03 (0.82–1.28)	1.10 (0.89–1.35)	**0.77 (0.62–0.97)***

Hazard ratios (HRs) with 95% confidence intervals (CIs) s) were derived from Cox proportional hazard models. Significant associations are shown in bold.
Model 1: crude model; Model 2: model 1 + age, sex; Model 3: model 2 + family history of diabetes; Model 4: model 3 + smoking, alcohol consumption, body mass index, hypertension, high-sensitivity C-reactive protein, and use of lipid-lowering medication; Model 5a: model 4 + fasting plasma glucose; Model 5b: model 4 + homeostatic model assessment of insulin resistance; Model 6a: model 5a + low-density lipoprotein cholesterol (LDL-C), triglycerides; Model 6b: model 5b + LDL-C, triglycerides.
Abbreviations: H5P, high-density lipoprotein 5 particles (10.3 nm); H6P, high-density lipoprotein 6 particles (10.8 nm); HDL, high-density lipoprotein; T2D, type 2 diabetes.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

HDL particles may convey protective effects on β-cells in vitro, allegedly by mediating cellular cholesterol efflux (CEC) and possibly also by its antioxidative function (42, 43). A positive correlation between CEC and large and medium HDL particles and HDL size, and an inverse correlation between CEC and small HDL particles were reported using J774 macrophages (44, 45). Although in another study CEC was only associated with the HDL particle concentration (46), this may link impaired HDL function to adiposity and/or insulin resistance coinciding with smaller HDL particles (23, 47). Another key process that could link T2D development with HDL function is the proprotein convertase subtilisin/kexine type 9 (PCSK9) pathway which may affect β-cell function by regulating cellular cholesterol homeostasis (48). PCSK9 also regulates sphingosine-1-phosphate, an important mediator of HDL function (49). In addition, the antioxidative function of HDL, an HDL metric possibly involved in maintaining β-cell function (43), is impaired in metabolic syndrome subjects after correction for HDL-C (24). Finally, HDL remodeling is at least in part consequence of changes in cholesteryl ester transfer protein (CETP) activity which in turn is affected by triglyceride-rich lipoproteins, and phospholipid transfer protein (PLTP) (40, 41, 46, 50, 51). Of note, recent studies suggest that administration of CETP inhibitors may decrease diabetes risk, but higher PLTP activity is associated with increased diabetes risk (52, 53). These findings raise the possibility that alterations in the HDL particle profile consequent to changes in CETP and PLTP could affect T2D development.

Secondary analyses suggested that large HDL particles and H6P were inversely associated with incident T2D in nonobese subjects but not in obese subjects. This could be interpreted as a result of altered HDL function in individuals with obesity and metabolic syndrome. Obesity and metabolic syndrome are not only associated with lower HDL-C and fewer large HDL particles but also with impaired HDL function exemplified as CEC (23, 54), consistent with dysfunctional HDL (55). In support of this, 2 randomized controlled studies found a beneficial effect of weight loss surgery on improving HDL function in patients

with obesity (56, 57). Also, serum paraoxonase-1 (PON-1), an antioxidative enzyme which predominantly associates with large HDL particles (58) and relates to PLTP activity (59), is impaired in metabolic syndrome subjects (60). However, paraoxonase-1 activity did not predict T2D development, making the role of this antioxidative enzyme in T2D development uncertain (61).

The association between HDL-C and HDL particles with development of T2D have been reported to vary with sex. Higher levels of HDL-C represented a protective marker of incident T2D in women but not in men (62, 63). Additionally, higher large HDL particles, as well as greater HDL size but reduced small HDL particles, were protective against T2D in 2 previous studies in women (17, 64), consistent with our findings showing an inverse association between large HDL particles and H6P with incident T2D in women but not in men. Furthermore, higher HDL-C and higher concentration of large HDL particles in women compared with men (25, 26) could be explained by different sex hormone effects on HDL metabolism. While exogenous estrogen increases predominately large HDL, testosterone may decrease large HDL (65, 66). Hence, we confirm that decreased large HDL particles and subspecies (H6P) may be risk markers of for T2D development especially in women.

A strength of the current study is that it included a large number of participants with a varied age range and with a follow-up period of 7.3 years. Furthermore, in sensitivity analysis applying the proportion of HDL subspecies, the inverse association of H4P and the positive association of H2P with incident T2D remained, significant, in fully adjusted analyses. A limitation of our study is that the majority of the participants were of White ethnic background, limiting extrapolation of our findings to other ethnicities. In this regard, it may be of importance that the inverse association of HDL-C and HDL-P with cardiovascular disease is absent in Black people (67).

In conclusion, HDL size is associated inversely with T2D risk in the general population. Among HDL subspecies, H4P was associated with a lower risk of T2D development, whereas higher levels of H2P were associated with a higher risk of future T2D. In addition, large HDL particles and H6P were associated with a lower risk of incident T2D in nonobese subjects but not in obese individuals. Of further note, large HDL particles and H6P were associated with a lower risk of incident T2D in women but not in men.

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Additional Information

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References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. 2017;**128**:40–50.
2. Lindström J, Peltonen M, Eriksson JG, et al.; Finnish Diabetes Prevention Study (DPS). Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). *Diabetologia*. 2013;**56**(2):284-293.
3. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch Intern Med*. 2007;**167**(10):1068-1074.
4. Abbasi A, Corpeleijn E, Gansevoort RT, et al. Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: the PREVEND study. *J Clin Endocrinol Metab*. 2013;**98**(8):E1352-E1359.
5. Hu PL, Koh YL, Tan NC. The utility of diabetes risk score items as predictors of incident type 2 diabetes in Asian populations: An evidence-based review. *Diabetes Res Clin Pract*. 2016;**122**:179-189.
6. Seo MH, Bae JC, Park SE, et al. Association of lipid and lipoprotein profiles with future development of type 2 diabetes in nondiabetic Korean subjects: a 4-year retrospective, longitudinal study. *J Clin Endocrinol Metab*. 2011;**96**(12):E2050-E2054.
7. Tabara Y, Arai H, Hirao Y, et al.; Nagahama study group. Different inverse association of large high-density lipoprotein subclasses with exacerbation of insulin resistance and incidence of type 2 diabetes: The Nagahama study. *Diabetes Res Clin Pract*. 2017;**127**:123-131.
8. Hwang YC, Hayashi T, Fujimoto WY, et al. Differential association between HDL subclasses and the development of type 2 diabetes in a prospective study of Japanese Americans. *Diabetes Care*. 2015;**38**(11):2100-2105.
9. Drew BG, Rye KA, Duffy SJ, Barter P, Kingwell BA. The emerging role of HDL in glucose metabolism. *Nat Rev Endocrinol*. 2012;**8**(4):237-245.

10. Kontush A, Lindahl M, Lhomme M, Calabresi L, Chapman MJ, Davidson WS. Structure of HDL: particle subclasses and molecular components. *Handb Exp Pharmacol*. 2015;**224**:3-51.
11. Camont L, Lhomme M, Rached F, et al. Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospholipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. *Arterioscler Thromb Vasc Biol*. 2013;**33**(12):2715-2723.
12. Zhu X, Parks JS. New roles of HDL in inflammation and hematopoiesis. *Annu Rev Nutr*. 2012;**32**:161-182.
13. Shalaurova I, Connelly MA, Garvey WT, Otvos JD. Lipoprotein insulin resistance index: a lipoprotein particle-derived measure of insulin resistance. *Metab Syndr Relat Disord*. 2014;**12**(8):422-429.
14. Flores-Guerrero JL, Connelly MA, Shalaurova I, et al. Lipoprotein insulin resistance index, a high-throughput measure of insulin resistance, is associated with incident type II diabetes mellitus in the Prevention of Renal and Vascular End-Stage Disease study. *J Clin Lipidol*. 2019;**13**(1):129-137.e1.
15. Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes*. 2003;**52**(2):453-462.
16. Festa A, Williams K, Hanley AJ, et al. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study. *Circulation*. 2005;**111**(25):3465-3472.
17. Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. *Diabetes*. 2010;**59**(5):1153-1160.
18. Mackey RH, Mora S, Bertoni AG, et al. Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis. *Diabetes Care*. 2015;**38**(4):628-636.
19. Hodge AM, Jenkins AJ, English DR, O'Dea K, Giles GG. NMR-determined lipoprotein subclass profile predicts type 2 diabetes. *Diabetes Res Clin Pract*. 2009;**83**(1):132-139.
20. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. HDL particle number measured on the Vantera®, the first clinical NMR analyzer. *Clin Biochem*. 2015;**48**(3):148-155.
21. Davidson WS, Shah AS. High-density lipoprotein subspecies in health and human disease: focus on type 2 diabetes. *Methodist Debakey Cardiovasc J*. 2019;**15**(1):55-61.
22. Sokooti S, Szili-Torok T, Flores-Guerrero JL, et al. High-density lipoprotein particles and their relationship to posttransplantation diabetes mellitus in renal transplant recipients. *Biomolecules*. 2020;**10**(3):1–14.
23. Annema W, Dikkers A, de Boer JF, et al. Impaired HDL cholesterol efflux in metabolic syndrome is unrelated to glucose tolerance status: the CODAM study. *Sci Rep*. 2016;**6**:27367.
24. Dullaart RP, de Boer JF, Annema W, Tietge UJ. The inverse relation of HDL anti-oxidative functionality with serum amyloid a is lost in metabolic syndrome subjects. *Obesity (Silver Spring)*. 2013;**21**(2):361-366.
25. Freedman DS, Otvos JD, Jeyarajah EJ, et al. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem*. 2004;**50**(7):1189-1200.
26. Eapen DJ, Kalra GL, Rifai L, Eapen CA, Merchant N, Khan BV. Raising HDL cholesterol in women. *Int J Womens Health*. 2009;**1**(1):181-191.
27. Sattar N. Gender aspects in type 2 diabetes mellitus and cardiometabolic risk. *Best Pract Res Clin Endocrinol Metab*. 2013;**27**(4):501-507.
28. Lambers Heerspink HJ, Brantsma AH, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort RT; PREVEND Study Group. Albuminuria assessed from first-morning-void urine samples versus 24-hour urine collections as a predictor of cardiovascular morbidity and mortality. *Am J Epidemiol*. 2008;**168**(8):897-905.
29. Abbasi A, Corpeleijn E, Postmus D, et al. Plasma procalcitonin and risk of type 2 diabetes in the general population. *Diabetologia*. 2011;**54**(9):2463-2465.
30. Gruppen EG, Connelly MA, Sluiter WJ, Bakker SJL, Dullaart RPF. Higher plasma GlycA, a novel pro-inflammatory glycoprotein biomarker, is associated with reduced life expectancy: The PREVEND study. *Clin Chim Acta*. 2019;**488**:7-12.
31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;**28**(7):412-419.
32. Kunutsor SK, Bakker SJ, Kootstra-Ros JE, Blokzijl H, Gansevoort RT, Dullaart RP. Inverse linear associations between liver aminotransferases and incident cardiovascular disease risk: The PREVEND study. *Atherosclerosis*. 2015;**243**(1):138-147.
33. Berends AMA, Buitenwerf E, Gruppen EG, et al. Primary aldosteronism is associated with decreased low-density and high-density lipoprotein particle concentrations and increased GlycA, a pro-inflammatory glycoprotein biomarker. *Clin Endocrinol (Oxf)*. 2019;**90**(1):79-87.
34. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med*. 2006;**26**(4):847-870.
35. Sokooti S, Flores-Guerrero LL, Kieneker LM, et al. HDL particle subspecies and their association with incident type 2 diabetes: the PREVEND study. *Figshare*. Deposited October 8, 2020. https://doi.org/10.6084/m9.figshare.13567832.v3
36. Connelly MA, Shalaurova I, Otvos JD. High-density lipoprotein and inflammation in cardiovascular disease. *Transl Res*. 2016;**173**:7-18.
37. Davidson WS, Heink A, Sexmith H, et al. Obesity is associated with an altered HDL subspecies profile among adolescents with metabolic disease. *J Lipid Res*. 2017;**58**(9):1916-1923.
38. Goff DC Jr, D'Agostino RB Jr, Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. *Metabolism*. 2005;**54**(2):264-270.
39. Gordon SM, Deng J, Lu LJ, Davidson WS. Proteomic characterization of human plasma high density lipoprotein fractionated by gel filtration chromatography. *J Proteome Res*. 2010;**9**(10):5239-5249.
40. Dallinga-Thie GM, Dullaart RP, van Tol A. Derangements of intravascular remodeling of lipoproteins in type 2 diabetes mellitus: consequences for atherosclerosis development. *Curr Diab Rep*. 2008;**8**(1):65-70.
41. Borggreve SE, De Vries R, Dullaart RP. Alterations in high-density lipoprotein metabolism and reverse cholesterol transport in

insulin resistance and type 2 diabetes mellitus: role of lipolytic enzymes, lecithin:cholesterol acyltransferase and lipid transfer proteins. *Eur J Clin Invest*. 2003;**33**(12):1051-1069.
42. von Eckardstein A, Widmann C. High-density lipoprotein, beta cells, and diabetes. *Cardiovasc Res*. 2014;**103**(3):384-394.
43. Dullaart RP, Annema W, de Boer JF, Tietge UJ. Pancreatic β-cell function relates positively to HDL functionality in well-controlled type 2 diabetes mellitus. *Atherosclerosis*. 2012;**222**(2):567-573.
44. Koekemoer AL, Codd V, Masca NGD, et al. Large-scale analysis of determinants, stability, and heritability of high-density lipoprotein cholesterol efflux capacity. *Arterioscler Thromb Vasc Biol*. 2017;**37**(10):1956-1962.
45. Mutharasan RK, Thaxton CS, Berry J, et al. HDL efflux capacity, HDL particle size, and high-risk carotid atherosclerosis in a cohort of asymptomatic older adults: the Chicago Healthy Aging Study. *J Lipid Res*. 2017;**58**(3):600-606.
46. Rohatgi A, Khera A, Berry JD, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med*. 2014;**371**(25):2383-2393.
47. Hernáez Á, Soria-Florido MT, Schröder H, et al. Role of HDL function and LDL atherogenicity on cardiovascular risk: a comprehensive examination. *Plos One*. 2019;**14**(6):e0218533.
48. Da Dalt L, Ruscica M, Bonacina F, et al. PCSK9 deficiency reduces insulin secretion and promotes glucose intolerance: the role of the low-density lipoprotein receptor. *Eur Heart J*. 2019;**40**(4):357-368.
49. Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. Obesity and dyslipidemia. *Metabolism*. 2019;**92**:71-81.
50. Dallinga-Thie GM, Dullaart RP, van Tol A. Concerted actions of cholesteryl ester transfer protein and phospholipid transfer protein in type 2 diabetes: effects of apolipoproteins. *Curr Opin Lipidol*. 2007;**18**(3):251-257.
51. Vergeer M, Boekholdt SM, Sandhu MS, et al. Genetic variation at the phospholipid transfer protein locus affects its activity and high-density lipoprotein size and is a novel marker of cardiovascular disease susceptibility. *Circulation*. 2010;**122**(5):470-477.
52. Masson W, Lobo M, Siniawski D, et al. Therapy with cholesteryl ester transfer protein (CETP) inhibitors and diabetes risk. *Diabetes Metab*. 2018;**44**(6):508-513.
53. Abbasi A, Dallinga-Thie GM, Dullaart RP. Phospholipid transfer protein activity and incident type 2 diabetes mellitus. *Clin Chim Acta*. 2015;**439**:38-41.
54. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJR. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar HDL-C to remove cholesterol. *Arter Thromb Vasc Biol*. 2010;**30**(4):796–801.
55. Acuña-Alonzo V, Flores-Dorantes T, Kruit JK, et al. A functional ABCA1 gene variant is associated with low HDL-cholesterol levels and shows evidence of positive selection in Native Americans. *Hum Mol Genet*. 2010;**19**(14):2877-2885.
56. Davidson WS, Inge TH, Sexmith H, et al. Weight loss surgery in adolescents corrects high-density lipoprotein subspecies and their function. *Int J Obes (Lond)*. 2017;**41**(1):83-89.
57. Lorkowski SW, Brubaker G, Rotroff DM, et al. Bariatric surgery improves hdl function examined by apoa1 exchange rate and cholesterol efflux capacity in patients with obesity and type 2 diabetes. *Biomolecules*. 2020;**10**(4):1-13.
58. Dullaart RP, Otvos JD, James RW. Serum paraoxonase-1 activity is more closely related to HDL particle concentration and large HDL particles than to HDL cholesterol in Type 2 diabetic and non-diabetic subjects. *Clin Biochem*. 2014;**47**(12):1022-1027.
59. Dullaart RPF, Gruppen EG, Dallinga-Thie GM. Paraoxonase-1 activity is positively related to phospholipid transfer protein activity in type 2 diabetes mellitus: Role of large HDL particles. *Clin Biochem*. 2016;**49**(6):508-510.
60. Kappelle PJ, Bijzet J, Hazenberg BP, Dullaart RP. Lower serum paraoxonase-1 activity is related to higher serum amyloid a levels in metabolic syndrome. *Arch Med Res*. 2011;**42**(3):219-225.
61. Kunutsor SK, Kieneker LM, Bakker SJL, James RW, Dullaart RPF. Incident type 2 diabetes is associated with HDL, but not with its anti-oxidant constituent—paraoxonase-1: the prospective cohort PREVEND study. *Metabolism*. 2017;**73**:43-51.
62. Njolstad I, Arnesen EL-LP. Re: sex differences in risk factors for clinical diabetes mellitus in a general population: A 12-year follow-up of the Finnmark study [2]. *Am J Epidemiol*. 1999;**149**(11):1073-1074.
63. Fagot-Campagna A, Narayan KM, Hanson RL, et al. Plasma lipoproteins and incidence of non-insulin-dependent diabetes mellitus in Pima Indians: protective effect of HDL cholesterol in women. *Atherosclerosis*. 1997;**128**(1):113-119.
64. Fagot-Campagna A, Knowler WC, Narayan KM, Hanson RL, Saaddine J, Howard BV. HDL cholesterol subfractions and risk of developing type 2 diabetes among Pima Indians. *Diabetes Care*. 1999;**22**(2):271-274.
65. Sacks FM, Walsh BW. Sex hormones and lipoprotein metabolism. *Curr Opin Lipidol*. 1994;**5**(3):236-240.
66. Asscheman H, Gooren LJ, Megens JA, Nauta J, Kloosterboer HJ, Eikelboom F. Serum testosterone level is the major determinant of the male-female differences in serum levels of high-density lipoprotein (HDL) cholesterol and HDL2 cholesterol. *Metabolism*. 1994;**43**(8):935-939.
67. Singh K, Chandra A, Sperry T, et al. Associations between high-density lipoprotein particles and ischemic events by vascular domain, sex, and ethnicity: a pooled cohort analysis. *Circulation*. 2020:657-669. doi:10.1161/CIRCULATIONAHA.120.045713.