Thromboinflammation in high-risk human populations

Yuan, L.

CHAPTER 3

Altered HDL composition is associated with risk for complications in type 2 diabetes mellitus in South Asian descendants: a cross-sectional, case-control study on lipoprotein subclass profiling

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Abstract

Background: Composition of high-density lipoproteins (HDL) is emerging as an important determinant in the development of microvascular complications in type 2 diabetes mellitus (T2DM). Dutch South Asian (DSA) individuals with T2DM display an increased risk of microvascular complications compared to Dutch white Caucasian (DwC) individuals with T2DM. In this study, we aimed to investigate whether changes in HDL composition associate with increased microvascular risk in this ethnic group and lead to new lipoprotein biomarkers.

Methods: Using ^{1}H nuclear magnetic resonance (NMR) spectroscopy and Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA^{TM}) software, plasma lipoprotein changes were determined in 51 healthy individuals (30 DwC, 21 DSA) and 92 individuals with T2DM (45 DwC, 47 DSA) in a cross-sectional, case-control study. Differential HDL subfractions were investigated using multinomial logistic regression analyses, adjusting for possible confounders including BMI and diabetes duration.

Results: We identified HDL compositional differences between healthy and diabetic individuals in both ethnic groups. Specifically, levels of ApoA2 and HDL-4 subfractions were lower in DSA compared to DwC with T2DM. ApoA2 and HDL-4 subfractions also negatively correlated with waist circumference, waist-to-hip ratio, HbA1c, glucose levels, and disease duration in DSA with T2DM, and associated with increased incidence of microvascular complications.

Conclusion: While HDL composition differed between controls and T2DM in both ethnic groups, the lower levels of lipid content in the smallest HDL subclass (HDL-4) in DSA with T2DM appeared to be more clinically relevant, with higher odds of having diabetes-related pan-microvascular complications such as retinopathy and neuropathy. These typical differences in HDL could be used as ethnicity-specific T2DM biomarkers.

Keywords: High-density lipoprotein composition, NMR, Dutch South Asians, type 2 diabetes mellitus, microvascular complications

Introduction

The worldwide rising prevalence of type 2 diabetes mellitus (T2DM) is one of the major challenges to public health in the 21st century [1]. Being overweight and obese are the most prominent risk factors for T2DM. However, South Asians (SA) have a significantly higher risk of developing T2DM compared to white Caucasians (wC) even with a body mass index (BMI) of around 23, which strongly suggests that different pathophysiological pathways in this particular ethnic group may lead to T2DM progression [2]. Additionally, compared to other ethnic groups, SAs tend to develop T2DM 5 to 10 years earlier [2], and SA patients with T2DM are more prone to develop microvascular complications such as diabetic retinopathy, with a faster progression and greater disease severity compared to West European patients with T2DM [3-5].

Previous studies showed that since the reduction of in particular low-density lipoproteins (LDL) by statins was associated with insulin resistance and increased risk of hyperglycaemic complications in T2DM, high-density lipoproteins (HDL) may be a better biomarker in diabetes progression [6-8]. HDL is composed of various lipids and proteins including apolipoproteins, enzymes, and lipid transfer factors, and exhibit large heterogeneity in size, density, and composition. The various HDL fractions have different functionalities with respect to mediating cholesterol efflux, anti-oxidation, anti-inflammation, and anti-thrombotic processes [9,10]. Low plasma HDL cholesterol (HDL-C) levels, for instance, were found associated with an increased risk of T2DM and cardiovascular disease [11-13], while also the importance of HDL functionality to cardiovascular disease incidence and prognosis of heart failure was observed [14-16], one of the main complications among T2DM patients. Emerging evidence further indicated that HDL particle quality has a causative impact on diabetes [17-20].

The majority of these studies were performed in Western populations, however, very little is known about HDL functional changes in individuals with T2DM from other ethnic groups. Given that SA represent a unique high-risk population with different pathophysiological

pathways to T2DM progression, dysfunction of HDL might be an important aspect overlooked in this population. Therefore, we hypothesized that specific differences in HDL composition in Dutch SA (DSA) with T2DM relate to their increased vulnerability to diabetes-related microvascular complications. In the present study, we aimed to investigate whether differences in plasma HDL composition in DSA with T2DM in relation to Dutch wC (DwC) with T2DM and their respective non-diabetic controls are associated with diabetes-related microvascular complications to provide possible new biomarkers. For this, we used ^{1}H nuclear magnetic resonance (NMR) spectroscopy and the validated Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA^{TM}) software [21-26] to measure plasma HDL subclass and lipid concentrations for HDL composition determination in DSA individuals with and without T2DM, compared with DwC without or with T2DM.

Material and methods

Study population. For the present cross-sectional study, baseline samples were used from the MAGNetic resonance Assessment of VICTOza efficacy in the Regression of cardiovascular dysfunction in type 2 diAbetes mellitus (MAGNA VICTORIA) study from two previous randomized controlled trials (RCT, ClinicalTrials.gov NCT01761318 [27] and NCT02660047 [28], respectively), together with age and gender matched healthy controls from both ethnic groups [29]. In the first trial, 50 DwC with T2DM were recruited while 51 DSA with T2DM were recruited in the second trial. T1DM patients were excluded in both trials. One DwC-T2DM and four DSA-T2DM patients withdrew from the RCTs. Healthy controls were recruited by advertisement in Leiden University Medical Center (Leiden, The Netherlands) and local newspapers, as mentioned elsewhere [29]. Additionally, 51 healthy non-diabetic control participants (30 DwC-C and 21 DSA-C) were prospectively enrolled with a similar age and sex distribution to the corresponding T2DM patients. Ethnicity was based on the self-identified and self-reported biological parents' and ancestors' origins. Participants with complete informed consent were included. The study conformed to the

revised Declaration of Helsinki and ethical approval was obtained from the Institutional Review Board (Leiden University Medical Center, Leiden, the Netherlands).

For the present study, we excluded participants of whom plasma samples were not available.

Sample preparation for ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Sample preparation was performed consistently with the requirements of the Bruker B.I.LISA lipoprotein analysis protocol. The EDTA plasma samples were thawed at room temperature and immediately homogenized by inverting the tubes 10 times. Next, 200 µL of plasma was manually transferred to a Ritter 96 deep-well plate. A Gilson 215 liquid handler robot was used to mix 120 µL of plasma with 120µL, 75 mM disodium phosphate buffer in H_2O/D_2O (80/20) with a pH of 7.4 containing 6.15 mM NaN_3 and 4.64 mM sodium 3-[trimethylsilyl] d4-propionate (Cambridge Isotope Laboratories). Using a modified second Gilson 215 liquid handler, 190 µL of each sample was transferred into 3 mm Bruker SampleJet NMR tubes. Subsequently, the tubes were sealed by POM ball insertion and transferred to the SampleJet autosampler where they were kept at 6°C while queued for acquisition.

NMR spectroscopy measurement and processing. All proton nuclear magnetic resonance (^{1}H-NMR) experiments were acquired on a 600 MHz Bruker Avance Neo spectrometer (Bruker Corporation, Billerica, USA) equipped with a 5-mm triple resonance inverse (TCI) cryogenic probe head with a Z-gradient system and automatic tuning and matching.

The NMR spectra were acquired following the Bruker B.I.Methods protocol. Before the measurements, a standard 3 mm sample of 99.8% methanol-d4 (Bruker) was used for temperature calibration [30]. A standard 3 mm QuantRefC sample (Bruker) was measured as the quantification reference and for quality control. All experiments were recorded at 310 K. The duration of the π/2 pulses was automatically calibrated for each sample using a

homonuclear-gated nutation experiment on the locked and shimmed samples after automatic tuning and matching of the probe head [31]. For water suppression, presaturation of the water resonance with an effective field of γB1 = 25 Hz was applied during the relaxation delay and the mixing time of the NOESY1D experiment [32].

The NOESY1D experiment was recorded using the first increment of a NOESY pulse sequence with a relaxation delay of 4 s and a mixing time of 10 ms [33]. After applying 4 dummy scans, 32 scans of 98,304 points covering a sweep width of 17,857 Hz were recorded.

The lipoprotein values were extracted from the NOESY1D plasma spectra by submitting the data to the commercial Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA) platform [21-26]. This approach extracts information about lipoproteins and lipoprotein subfractions in plasma. In the current study, we focused on the HDL particles; dissected into four subclasses (sorted according to increasing density and decreasing size; i.e., HDL-1, HDL-2, HDL-3, and HDL-4), component concentration and composition. Lipid content including total cholesterol, free cholesterol, phospholipids, and triglycerides within total HDL and HDL subclasses represents the composition of HDL, called HDL main fractions and subfractions. The calculated esterified cholesterol was not included in the present study.

Isolation of primary human umbilical vein endothelial cells (HUVEC). Primary HUVECs were isolated from human umbilical cords obtained at the Leiden University Medical Center after written informed consent was obtained and the umbilical cord was collected and processed anonymously. The umbilical cord was rinsed with PBS to remove any remaining blood. To detach the endothelial cells from the umbilical cord, trypsin/EDTA (BE17-161E, Lonza, B-4800 Verviers, Belgium) was used. After 20 minutes of incubation at 37°C, the trypsin was inactivated with 20% FCS in PBS, and the entire solution in the umbilical cord was collected. The umbilical vein was flushed with PBS one more time to ensure that all detached cells were collected before centrifugation at 1200 rpm for 7 minutes. Cells were

then cultured in 1% gelatin pre-coated flasks in endothelial growth medium (EGM2 medium, C-22011 supplemented with SupplementMix, Promocell, Heidelberg, Germany) with 1% antibiotics (penicillin/streptomycin, 15070063, Gibco, Paisley, UK). We cultured HUVECs from two different donors (one boy and one girl). We pooled them together and froze them in several vials for future experiments once they were confluent.

HDL isolation. Leftover plasma samples from ^{1}H NMR measurements were used to isolate HDL based on the protocol from a previous study by Mulder *et al.* [34]. First, a 1:2 mixture of 36% polyethylene glycol (PEG 6000, 442714K, VWR International, Lutterworth, UK) in 10 mM HEPES (pH 8, H3375, Sigma Aldrich, the Netherlands) and plasma was prepared. Following that, samples were incubated for 30 min on ice to precipitate ApoB-containing lipoproteins, and centrifuged for 30 minutes at 2200 g. The HDL-containing supernatants were collected, kept at 4°C, and used the following day for HDL functionality tests.

HDL anti-thrombotic capacity. Primary HUVECs were used to test HDL's anti-thrombotic properties. In the 96-well plate, HUVECs (passage 3) were seeded at a density of 4×10^{5} cells/well. The following day, HUVECs were pre-incubated for 30 min with 2% ApoB-depleted plasma or an equal volume of precipitation reagent in HEPES as a control. Tumor necrosis factor α (TNF-α, H8916, Sigma Aldrich, the Netherlands) was then added at a concentration of 10 ng/mL. After another 5 h of incubation, the cell surface was washed once with HBSS, no calcium, and no magnesium (14170120, Gibco™, Paisley, UK). Each well received 50 µL of normal pooled plasma before being placed in the Fluorometer for a 10-min incubation at 37°C. The formation of thrombin was started by mixing 10 µL of the fluorogenic substrate with calcium (TS50.00 FluCa-kit; Thrombinoscope BV, Maastricht, the Netherlands). The final reaction volume was 60 µL. Thrombin formation was measured every 20 sec for 60 min and calibrated using Thrombinoscope software (Supplemental

Figure S4a). To assess HDL anti-thrombotic capacity, endogenous thrombin potential (ETP) was measured. To limit potential variation due to different plate conditions, HDL anti-thrombotic capacity measurements were performed at the same time using the same batch of pooled HUVECs and reagents. For each individual, measurements were taken in three technical replicates. To avoid batch effects, each plate contains samples from four groups.

Statistical analyses. Differences in baseline characteristics were tested between 4 groups, namely DwC healthy controls (DwC-C), DwC-T2DM, DSA healthy controls (DSA-C), and DSA-T2DM. Categorical variables were expressed as total number (percentage, %) and differences between groups were tested with the Chi-square test or Fisher's exact test. Normally distributed continuous variables were expressed as mean (standard deviation, SD), and differences were evaluated by post hoc tests of unpaired One-way ANOVA test; skewed continuous variables were presented as median (25-75 percentile) and differences were assessed using the Kruskal-Wallis test.

Based on ^{1}H NMR spectroscopy, a total of 112 lipoprotein main fractions and subfractions were extracted. To confirm the reliability of the NMR measurements, correlation analyses were performed between clinical routine measurements and corresponding parameters from NMR. We first extracted concentrations of all lipoprotein subclasses and performed a Pearson's correlation between clinical routine measurements (total triglycerides, total cholesterol, LDL-cholesterol [LDL-C], and HDL-C) and corresponding parameters from NMR.

Four groups (DSA-T2DM, DSA-C, DwC-T2DM, and DwC-C) were considered as four outcomes, and concentrations of HDL main fractions and subfractions were scaled (z-normalization, i.e., with mean=0 and SD=1) to identify lipoproteins with different concentrations, and multinomial logistic regression analysis (MLR) was used. We set a specific reference for each comparison: between DSA-T2DM and DSA-C, DSA-C was the reference; between DwC-T2DM and DwC-C, DwC-C was the reference; between DSA-T2DM and DwC-T2DM, DwC-

T2DM was the reference; between DSA-C and DwC-C, DwC-C was the reference. The analyses were adjusted for several potential confounding factors. First, age (continuous variable), sex (dichotomous variable), and current smoking status (dichotomous variable) were adjusted for model 1. Second, model 1 was further adjusted for BMI (continuous variable) (model 2). Third, when comparing T2DM individuals from two ethnic groups, we set the disease duration of healthy individuals as zero and adjusted the diabetes duration (continuous variable) (model 3). We considered a p-value < 0.05 as significant. The results were expressed as regression coefficient (β), and odds ratios (ORs) with a 95% confidence interval (CI) to evaluate the differences between concentrations of HDL main fractions and subfractions and different groups. We followed the STROBE guidelines to report our findings.

Next, based on HDL main fractions and subfractions, supervised dimension reduction analysis called sparse partial least squares discriminant analysis (sPLS-DA) was performed by using the "mixOmics" package in R [35]. sPLS-DA enables the selection of the most predictive or discriminative features in the data to classify the samples. Thus, by using this analysis, we aimed to rank and validate the features according to their contribution between DSA-T2DM and DSA-C, DwC-T2DM and DwC-C, DSA-T2DM and DwC-T2DM, and DSA-C and DwC-C. We then compared the rank of features determined by sPLS-DA and those generated from multinomial logistic regression by using Spearman's correlation to further validate the findings. The contribution of loading features was calculated based on two components for each sPLS-DA model.

Pearson's correlation analyses were performed to reveal associations between differential HDL subfractions and laboratory markers including fasting levels of gamma-glutamyl transferase (GGT), haemoglobin A1c (HbA1c), and glucose and anthropometrics markers including visceral adipose tissue (VAT), waist circumference, and waist-to-hip ratio.

We then investigated the correlation between disease duration and differential HDL subfractions, and further examined the associations between HDL subfractions and pan-microvascular-related complications, which were defined as composite diabetes-related

complications including diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy. A T2DM participant with one or more of these complications was considered as having a pan-microvascular-related complication. This analysis was performed separately for DSA and DwC populations, and the Mann-Whitney-Wilcoxon test was used to assess the statistical differences between the cases (e.g., with pan-microvascular-related complications) and controls (e.g., without pan-microvascular-related complications) for each ethnic group. Statistical analyses were performed in R (version 4.1.0) and GraphPad Prism version 8 (Graphpad Inc., La Jolla, CA, USA).

Results

Clinical characteristics. After consecutive exclusion of one T1DM individual from the MAGNA VICTORIA study, 5 individuals who withdrew from the RCT, and 3 individuals with missing plasma samples, 92 individuals with T2DM were included. None of the healthy controls were excluded (Figure 1). In total, 143 individuals were included in the present study: 47 DSA-T2DM subjects (19 men/28 women), 21 DSA-C (9 men/15 women), 45 DwC-T2DM subjects (25 men/20 women), and 30 DwC-C (16 men/14 women), (Table 1).

Compared to DSA-C, systolic blood pressure, body surface area (BSA), BMI, waist circumference, waist-to-hip ratio, fasting glucose, HbA1c, triglyceride levels, and VAT were higher in DSA-T2DM; while total cholesterol, HDL-C, and LDL-C were lower (Table 1). In the DwC-T2DM group, BSA, BMI, waist circumference, waist-to-hip ratio, total cholesterol, LDL-C, total body fat, and SAT were higher compared to DwC-C, together with a higher proportion of current smokers. Comparing individuals with T2DM between the ethnic groups, DSA subjects had a longer diabetes duration, a higher incidence of vascular-related complications (e.g., retinopathy and macrovascular problems), and a higher albumin/creatine ratio compared to DwC subjects.

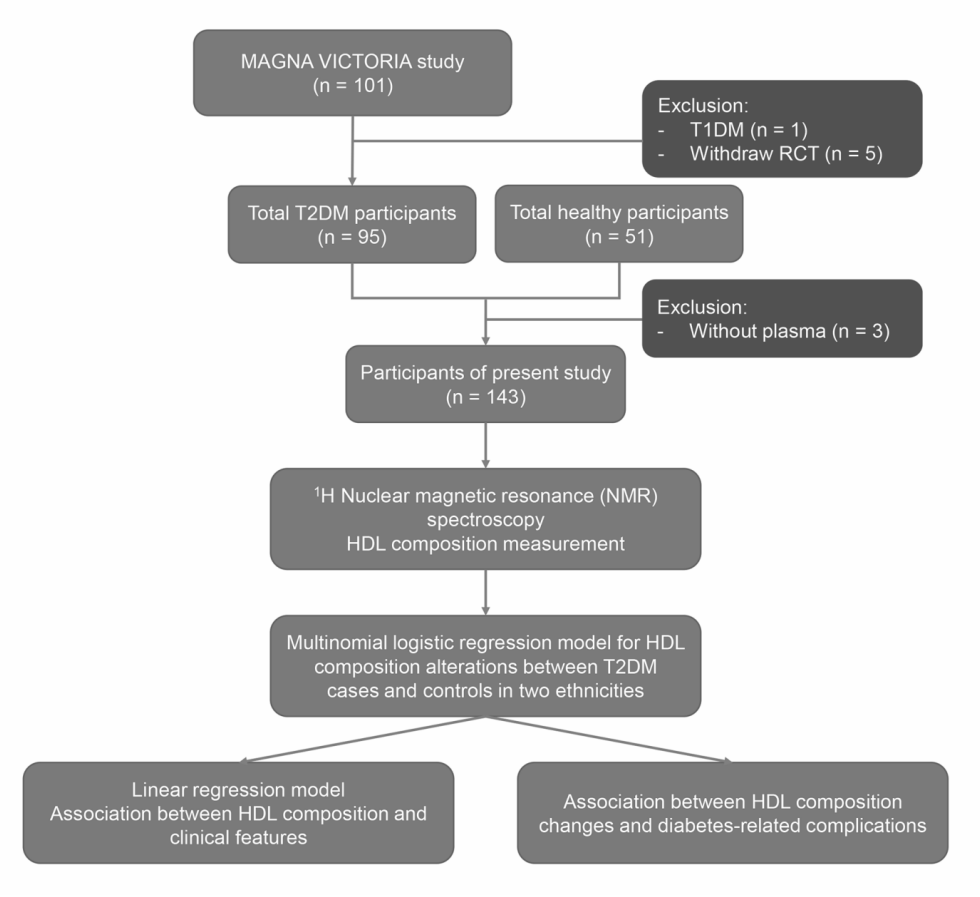


Figure 1. Flow chart of the study

Table 1. Clinical Characteristics of study participants.

	Dutch South Asian			Dutch white Caucasian			Dutch South Asian T2DM vs Dutch white Caucasian T2DM
	Control (n=21)	T2DM (n=47)	p-value[1]	Control (n=30)	T2DM (n=45)	p-value[1]	p-value[2]
Demographics							
Age (years)	48.3 (8.1)	54.9 (10.1)	0.0367	57.9 (7.9)	59.0 (6.5)	ns	ns
Women (no, %)	15 (71.4%)	28 (59.6%)	ns	14 (46.7%)	20 (44.4%)	ns	ns
Current smoker (no, %)	3 (14.3%)	7 (14.9%)	ns	1 (3.3%)	9 (20.0%)	0.0437	ns
Medical history diabetes							
Duration diabetes mellitus (years)	-	17.9 (10)	-	-	10.3 (6.0)	-	<0.0001
Nephropathy (n, %)	-	10 (21.3%)	-	-	11 (23.4%)	-	ns
Neuropathy (n, %)	-	14 (29.8%)	-	-	15 (31.9%)	-	ns
Retinopathy (n, %)	-	24 (51.1%)	-	-	5 (10.6%)	-	<0.0001
Macrovascular (n, %)	-	13 (27.7%)	-	-	2 (4.3%)	-	0.0037
Medication use			-			-	
Metformin (n, %)	-	45 (95.7%)	-	-	45 (100%)	-	ns
Sulfonylurea derivatives (n, %)	-	8 (17.0%)	-	-	13 (28.9%)	-	ns
Insulin (n, %)	-	36 (76.6%)	-	-	29 (64.4%)	-	ns
Anti-hypertensive medication (n, %)	-	34 (72.3%)	-	-	34 (75.6%)	-	ns
ACE-inhibitors (n, %)	-	13 (27.7%)	-	-	17 (37.8%)	-	ns
Statins (n, %)	-	36 (76.6%)	-	-	36 (80.0%)	-	ns
Blood pressure							
Systolic blood pressure (mmHg)	123.5 (13.7)	144.6 (21.5)	0.0003	126.2 (12.1)	141.3 (15.0)	0.0007	ns
Diastolic blood pressure (mmHg)	80.2 (11.8)	85.3 (10.0)	ns	80 (77-83)	86.9 (8.8)	0.0158	ns
Anthropometrics							
BSA, m^2	1.7 (0.2)	1.9 (0.2)	<0.0001	1.9 (0.2)	2.2 (0.2)	<0.0001	<0.0001
BMI (kg/m^2)	23.5 (3.0)	29.5 (4.0)	<0.0001	24.3 (3.3)	32.3 (3.9)	<0.0001	0.0018
Waist circumference, cm	82.0 (7.4)	101.0 (9.5)	<0.0001	86.6 (9.1)	110.4 (8.9)	<0.0001	<0.0001
Waist-to-hip ratio	0.9 (0.1)	1 (0.1)	<0.0001	0.9 (0.1)	1 (0.1)	<0.0001	0.0039
Laboratory markers							
Fasting glucose (mmol/L)	5.0 (0.3)	8.1 (3.0)	<0.0001	5.2 (0.5)	7.8 (2.1)	<0.0001	ns
HbA1c (mmol/mol)	35.5 (2.4)	67.8 (11.3)	<0.0001	35.5 (2.7)	64.9 (10.7)	<0.0001	ns
GGT (IU/L)	-	28 (18-37.5)	-	-	32 (21-45)	-	ns
Total cholesterol (mmol/L)	5.4 (0.8)	4.2 (0.9)	<0.0001	5.7 (1.1)	4.8 (1.0)	0.0018	0.0226
HDL-cholesterol (mmol/L)	1.6 (0.3)	1.2 (0.3)	0.0028	1.9 (0.5)	1.3 (0.3)	<0.0001	ns
LDL-cholesterol (mmol/L)	3.4 (0.7)	2.1 (0.8)	<0.0001	3.3 (1.0)	2.6 (0.8)	0.0017	0.0416
Triglycerides (mmol/L)	0.9 (0.3)	1.8 (1.4)	0.0031	0.9 (0.7-1.2)	2.1 (1.3)	<0.0001	ns
Serum creatinine (µmol/mL)	68.0 (60.0-79.0)	67.0 (59.0-83.5)	ns	73.0 (68.0-85.0)	68.0 (57.0-80.0)	ns	ns
Urinary markers							
Albumin/creatine ratio (mg/mmol)	-	2.7 (0.55-8.45)	-	-	0.7 (0-2.5)	-	0.0037
Micro-albuminuria (n, %)[a]	-	15 (31.9%)	-	-	7 (15.6%)	-	-
Macro-albuminuria (n, %)[b]	-	7 (14.9%)	-	-	1 (2.2%)	-	-
Radiology based markers							
Total body fat (%)	32.4 (7.1)	37.1 (9.1)	ns	32.5 (7.1)	37.2 (9.3)	<0.0001	ns
VAT, cm^2	73.2 (29.8)	166.4 (55.8)	<0.0001	74.7 (34.1)	205.6 (75.6)	<0.0001	ns
SAT, cm^2	233.2 (195.9-258.8)	300.1 (228.3-371.4)	0.0561	189.7 (148.8-238.6)	335.7 (262.4-419.5)	<0.0001	ns

Data are presented as mean (SD), median (25-75 percentile), or percentage.

Abbreviations: *ACE* Angiotensin-converting enzyme, *BMI* body mass index, *BSA* body surface area, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *ns* not-significant, *SAT* subcutaneous adipose tissue, *VAT* visceral adipose tissue.

[1]Post hoc tests of unpaired One-way ANOVA, Kruskal-Wallis test or Chi-square test, $p<0.05$

[2]Post hoc tests of unpaired One-way ANOVA or Kruskal-Wallis test, Chi-square test or Fisher's exact test, $p<0.05$

[a]Albumin-creatinine ratio between 3.0 – 30 mg/mmol. [b]Albumin-creatinine ratio > 30 mg/mmol

Most of these data have been published before [27-29].

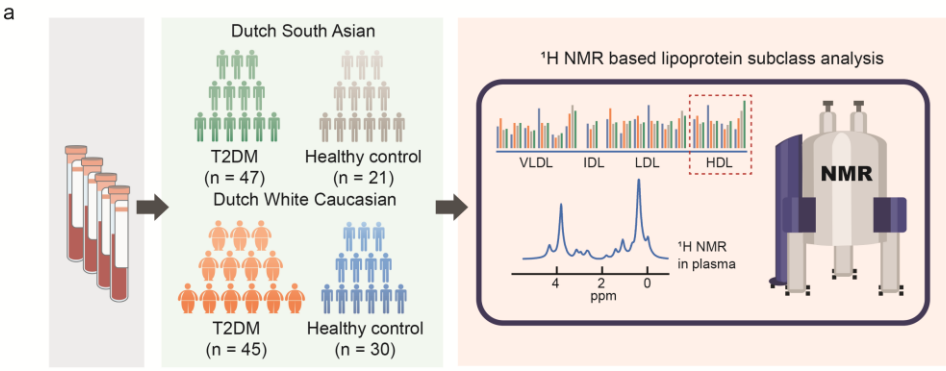
Comparison between NMR-based results vs clinical chemistry approach. NMR-based lipoprotein subclass measurements are shown in Figure 2a. To verify the quality of the NMR measurements we compared: total triglycerides, total cholesterol, HDL-C, and LDL-C to the clinical chemistry measurements revealing a high correlation in the total cohort (R = 0.81-0.99, p-value < 2e-16) as well as any single group (Supplemental Figure S1).

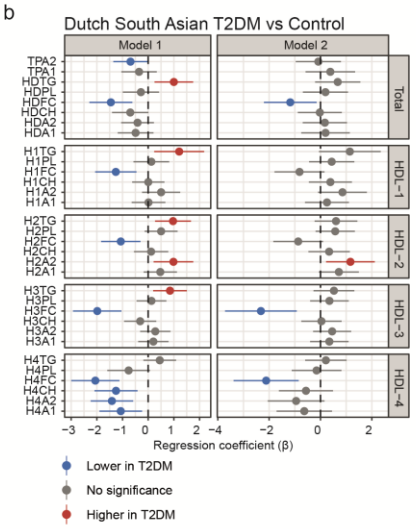
Different HDL compositions between healthy and diabetic individuals in two ethnic groups. To identify differences in HDL composition between the various groups, 32 HDL main fractions and subfractions were tested (Supplemental Table S1) using multinomial logistic regression analyses. Comparing healthy and diabetic individuals in Dutch South Asians, we found that 14 HDL subfractions were different (5 higher and 9 lower) in DSA-T2DM compared to DSA-C (Figure 2b and Supplemental Table S2, model 1); 21 HDL subfractions were different (4 higher and 17 lower) in DwC-T2DM compared to DwC-C (Figure 2c and Supplemental Table S3, model 1). After the adjustment of BMI (model 2), only 4 HDL subfractions remained significant (ApoA2 content in HDL-2 was higher and free cholesterol content in total HDL, HDL-3, and HDL-4 were lower) in DSA-T2DM (Figure 2b and Supplemental Table S2, model 2); 10 HDL subfractions persisted (triglyceride content in HDL-4 was higher and HDL-1 subfractions except H1TG and free cholesterol content in HDL were lower) in DwC-T2DM (Figure 2c and Supplemental Table S3, model 2). As multinomial logistic regression analysis was used to evaluate the association between HDL composition and the odds of having T2DM, the common trend in both ethnic groups showed that higher free cholesterol content in most HDL subclasses was associated with lower odds of having T2DM (Supplemental Table S2 and S3). Specifically, higher ApoA2 content in HDL-2 was associated with higher odds of having T2DM in DSA. In DwC, higher HDL-1 subfractions were associated with lower odds of having T2DM and higher triglyceride content in the smallest and dense HDL (i.e., HDL-4) was associated with higher odds of having T2DM (Supplemental Table S2 and S3).

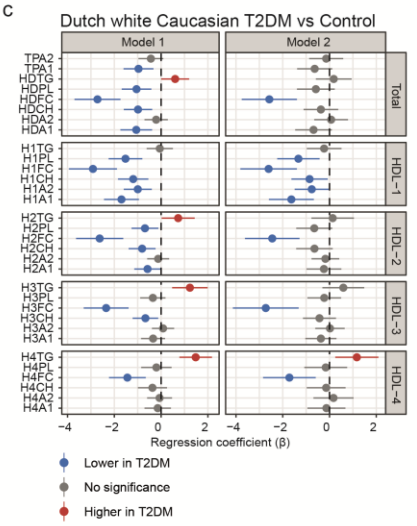
Furthermore, sPLS-DA, a supervised machine learning method combining variable selection and classification in a one-step procedure was performed by using HDL-related features. A clear separation between T2DM and healthy controls was observed in both ethnicities, and the top 5 ranked features in DSA-T2DM were free cholesterol content in total HDL and subclasses (HDL-2, HDL-3, and HDL-4) and ApoA2 in HDL-4 (HDFC, H2FC, H3FC, H4FC, and H4A2). Those in DwC-T2DM were free cholesterol content in total HDL and subclasses (HDL-1, HDL-2, and HDL-4) and ApoA1 in HDL1 (HDFC, H1FC, H2FC, H3FC, and H1A1), (Supplemental Figure S2c-d). The ranks generated from MLR and sPLS-DA tightly correlated in both ethnic groups (Supplemental Figure S2e-f). Our findings revealed ethnic differences in HDL composition between healthy and diabetic individuals that were not detectable by routine lipid or laboratory assessments.

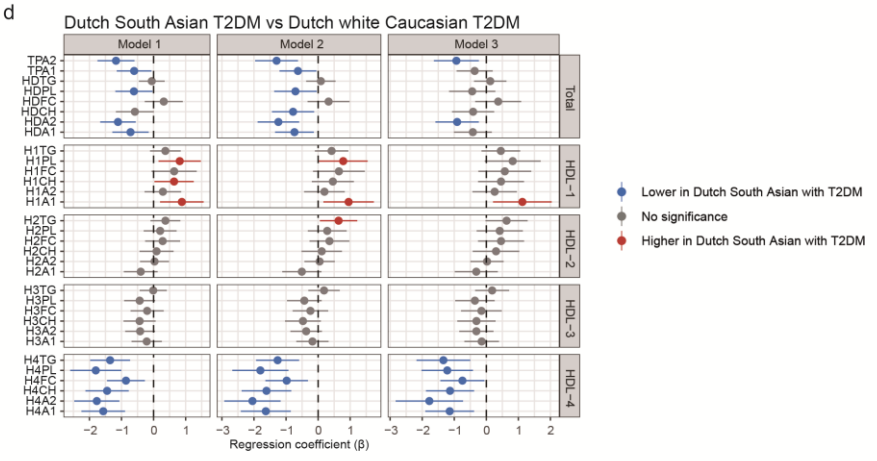
Figure 2. The ethnicity-specific distinction of HDL composition between Dutch South Asians and Dutch white Caucasians based on ^{1}H NMR. (a) Scheme of ^{1}H nuclear magnetic resonance in the present study. (b) Forest plot of differential lipoprotein subfractions between DSA-C and DSA-T2DM. (c) Forest plot of differential lipoprotein subfractions between DwC-C and DwC-T2DM. (d) Forest plot of differential lipoprotein subfractions between DSA-T2DM and DwC-T2DM. The regression coefficient and 95% confidence interval were depicted by a horizontal line with a dot. A non-significant association was represented by the color grey, a positive association was represented by the color red, and a substantial negative association was represented by the color blue. Model 1: adjusted age, gender, and current smoking status; Model 2 = model 1 + BMI; Model 3: model 2 + diabetes duration.

Abbreviations: A1: apolipoprotein A1; A2: apolipoprotein A2; CH: cholesterol; FC: free cholesterol; PL: phospholipid; TG: triglyceride. Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.


a
Dutch South Asian
T2DM
(n = 47)
Healthy control
(n = 21)
Dutch White Caucasian
T2DM
(n = 45)
Healthy control
(n = 30)
¹H NMR based lipoprotein subclass analysis
VLDL
IDL
LDL
HDL
NMR
¹H NMR
in plasma
4
2
0
ppm


b
Dutch South Asian T2DM vs Control
Model 1
Model 2
Total
HDL-1
HDL-2
HDL-3
HDL-4
Regression coefficient (β)
Lower in T2DM
No significance
Higher in T2DM


c
Dutch white Caucasian T2DM vs Control
Model 1
Model 2
Total
HDL-1
HDL-2
HDL-3
HDL-4
Regression coefficient (β)
Lower in T2DM
No significance
Higher in T2DM


d
Dutch South Asian T2DM vs Dutch white Caucasian T2DM
Model 1
Model 2
Model 3
Total
HDL-1
HDL-2
HDL-3
HDL-4
Regression coefficient (β)
Lower in Dutch South Asian with T2DM
No significance
Higher in Dutch South Asian with T2DM

HDL composition in plasma revealed ethnicity specificity in individuals with and without T2DM in two ethnic groups. When we compared T2DM subjects between DSA and DwC, notable differences were observed in HDL-4 subclass composition. Total ApoA1 and ApoA2, and phospholipid content in total HDL (HDPL) were lower in DSA-T2DM when compared to DwC-T2DM; whereas large HDL subclass composition such as ApoA1, cholesterol, and phospholipid content in HDL-1 (H1A1, H1CH, and H1PL) were higher in DSA-T2DM compared to DwC-T2DM (Supplemental Table S4 and Figure 2d, Model 1). After further adjustment for BMI and diabetes duration, the regression coefficient of differential HDL subfractions, albeit attenuated, remained different (Supplemental Table S4 and Figure 2d, Model 2 and Model 3), particularly the HDL-4 subclass composition and ApoA2 presence. Moreover, a significant difference in T2DM was observed between the two ethnic groups when using sPLS-DA (Figure 3a). Ranking HDL-related feature measurements by discriminating capability, we found that HDL-4 subclass composition and ApoA2 presence had the greatest contribution (Figure 3b). Notably, the rank generated from MLR and sPLS-DA highly correlated ($\rho = 0.97$, p-value < 2.2e-16, Figure 3c), which further validated our previous findings.

Considering the higher prevalence of T2DM in DSA, we also compared HDL composition among healthy individuals of both ethnicities and found that the majority of the HDL compounds were lower in DSAs, with only the triglyceride content in the HDL subclasses showing comparable distributions (Supplemental Table S5 and Supplemental Figure S3a). Meanwhile, similar but less profound findings generated from sPLS-DA were found in healthy individuals between the two ethnic groups (Supplemental Figure S2b-d). These results suggested that ApoA2 and the HDL-4 subclass composition were different in T2DM and had ethnic specificity.

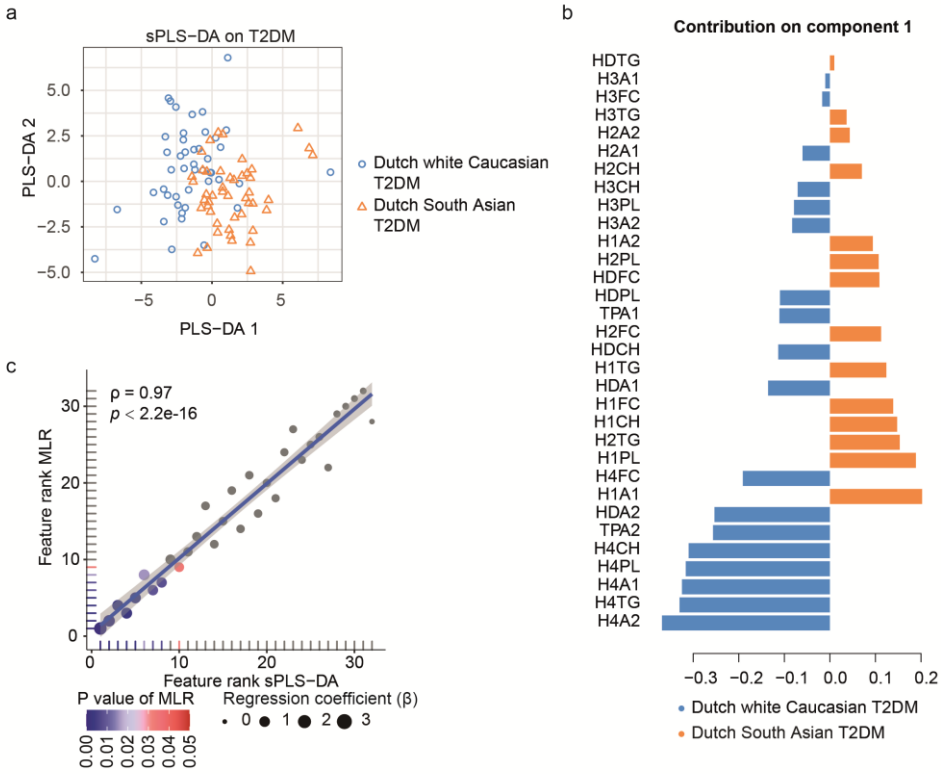


Figure 3. Sparse partial least squares discriminant analysis (sPLS-DA) differentiates DSA-T2DM from DwC-T2DM. (a) sPLS-DA performed on HDL main fractions and subfractions separates DSA-T2DM from DwC-T2DM. (b) Loading values of features with the contribution to differentiating DSA-T2DM from DwC-T2DM in the sPLS-DA. (c) Spearman's correlation between the rank generated from MLR and sPLS-DA. Dot color represents the p-value of MLR, and dot size represents the absolute value of regression coefficient.

Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.

HDL anti-thrombotic capacity reduction in T2DM in both ethnicities. Anti-thrombotic capacity was measured in 142 subjects to evaluate HDL functionality (47 DSA-T2DM, 21 DSA-C, 45 DwC-T2DM, and 29 DwC-C). Based on the thrombin generation curve, diabetic plasma generated more thrombin than non-diabetic plasma samples in both ethnic groups

(Supplemental Figure S4b and d). Furthermore, the endogenous thrombin potential (ETP) was significantly higher in T2DM patients versus healthy controls in both ethnicities (Supplemental Figure S4c and e), revealing that HDL functionality in anti-thrombin formation was impaired both in DSA and DwC with T2DM.

Associations between differential HDL subfractions and clinical outcomes. We further investigated the associations between differential HDL subfractions (ApoA2 and HDL-4 subclass composition) and clinical outcomes (laboratory and anthropometric markers). In DSA-T2DM, except H4TG, the other lipid content in HDL-4 subclass negatively correlated with waist-to-hip ratio and waist circumference; ApoA1 and ApoA2, cholesterol and phospholipid content in HDL-4 (H4A1, H4A2, H4CH, and H4PL) revealed a negative correlation with VAT; H4CH, H4FC, and H4PL negatively correlated with HbA1c and H4FC and H4PL negatively correlated with fasting glucose. Interestingly, H4A1 and H4A2 positively correlated with GGT (Supplemental Figure S5a).

In DwC-T2DM, TPA2 and HDA2 negatively correlated with waist-to-hip ratio; HDA2 and H4A2 correlated negatively with waist circumference while H4FC showed a negative correlation with fasting glucose levels (Supplemental Figure S5b). These results suggested that changes in HDL composition were clinically relevant, especially in DSA-T2DM which could partly reflect long-term dysregulated blood-glucose control.

Associations between differential HDL subfractions and pan-microvascular-related complications. As a longer disease duration may lead to a higher incidence of complications (Figure 4a and b), we examined the correlation between HDL subfractions and diabetes duration in cases of both ethnicities. Notably, we discovered that ApoA2 and all HDL-4 subfractions except H4TG negatively associated with diabetes duration in DSA-T2DM while we did not find these associations in DwC-T2DM (Figure 4c and d).

Given glycaemic control is associated with multiple diabetes-related complications, especially microvascular problems; we then investigated the associations between differential HDL subfractions (ApoA2 and HDL-4 subclass composition) and microvascular complications. Interestingly, there was no association between HDL subfractions and pan-microvascular-related complications in white Caucasian subjects with T2DM; while, in DSA-T2DM, we observed that total ApoA2, HDA2, H4A1, H4FC, and H4PL were lower in patients with pan-microvascular-related complications. When we compared single complications, TPA2, HDA2, H4A1, H4A2, and H4FC were significantly lower in DSA-T2DM with retinopathy, while H4FC was higher in DwC-T2DM with retinopathy (Figure 4b). In DSA-T2DM, TPA2, H4A1, H4A2, H4CH, H4FC, and H4PL were significantly lower in diabetic neuropathy while none of these HDL subfractions were associated with diabetic neuropathy in DwC-T2DM (Figure 4e and f). None of the differential HDL subfractions changed between groups with and without diabetic nephropathy. These data suggest that changes in HDL composition, especially TPA2 and HDL-4 subclass composition (except H4TG in DSA), were associated with diabetes-related microvascular complications such as neuropathy and retinopathy.

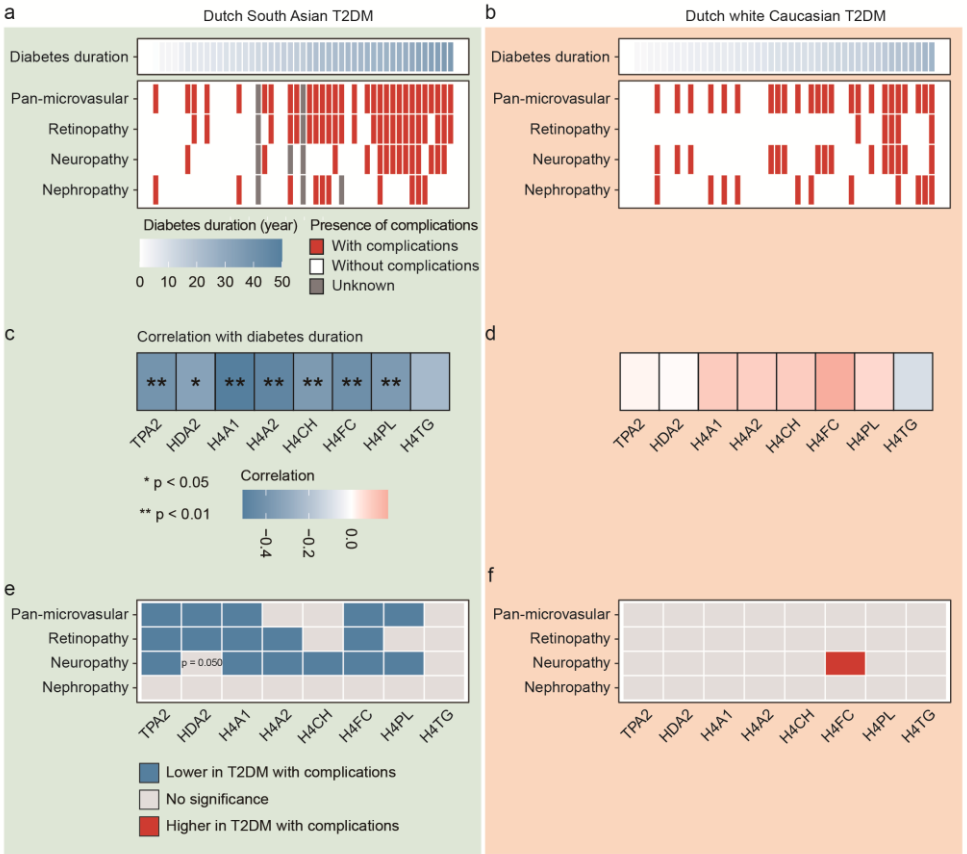


Figure 4. Associations between differential HDL subfractions and diabetes-related microvascular complications. (a) Association between disease duration and presence of diabetes-related microvascular complications in DSA-T2DM. (b) Association between disease duration and presence of diabetes-related microvascular complications in DwC-T2DM. (c) Pearson's correlation between diabetes duration and differential HDL subfractions in DSA-T2DM. (d) Correlation between diabetes duration and differential HDL subfractions in DwC-T2DM. Pearson's correlation analysis was performed. The color of scale bar represented Pearson's correlation R. *p<0.05; **p<0.01. (e) Summary heatmap showing the associations between differential HDL subfractions and diabetes-related microvascular complications in DSA-T2DM. (f) Summary heatmap showing the associations between differential HDL subfractions and diabetes-related microvascular complications in DSA-T2DM.

Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.

Discussion

In the present study, we observed HDL compositional differences between healthy and diabetic individuals in both ethnic groups. Specifically, we discovered that lower ApoA2 presence and HDL-4 subclass compositional changes in DSA individuals with T2DM were associated with a higher incidence of diabetes-related pan-microvascular complications such as retinopathy and neuropathy compared to DwC individuals with T2DM. These results indicated that differences in HDL composition might be used as an ethnicity-specific biomarker for DSA with T2DM and may provide a mechanism underlying the increased risk of microvascular complications in DSA.

Previous studies investigating HDL particle subspecies did not account for the HDL composition, particularly the lipid content, and the majority of these studies were conducted on white ethnic groups [36,37]. Only a single study showed HDL-2 and HDL-3 were associated with insulin resistance and beta cell function in South Asians at risk of T2DM [38]. In our study, we included both DSA and DwC populations and generated HDL main fractions and subfractions including 32 features, providing a higher resolution of HDL lipoprotein fraction than HDL-C alone. Sparse partial least squares discriminant analysis (sPLS-DA) based on HDL composition could not only distinguish healthy controls from T2DM in both ethnic groups but also show the distinction between T2DM in these two ethnic groups. This suggested that this technique may be clinically meaningful beyond the measurement of HDL-C, especially in different ethnicities.

We observed ethnicity-specific associations between HDL composition and T2DM and found a loss of large HDL subclass and higher triglycerides in the smallest HDL subclass in DwC with T2DM, consistent with the finding of the PREVEND study that HDL size is inversely associated with T2DM risk [36]. Interestingly, in DSA with T2DM, we observed an opposite trend that the lipid content in the smallest HDL subclass was specifically lower and triglycerides were higher in the largest HDL subclass when adjusting for age, gender, and current smoking status. After BMI adjustment, only free cholesterol content in HDL

persisted, suggesting that BMI plays a vital role in affecting HDL composition in DSA-T2DM and highlighting the importance of ethnic-specific guidelines for BMI. Furthermore, a randomised trail proved that a structured weight management programme incorporating a total diet replacement weight loss phase was acceptable to individuals of SA ethnicity and could achieve T2DM remissions similarly to other populations, indicating the favourable aspects of dietary weight-management in this high-risk population [39].

In our study, with individuals with T2DM of both ethnicities receiving similar medical care, we still discovered that total ApoA1 and ApoA2, as well as HDL-4 subclass composition, were significantly lower in DSA with T2DM than DwC with T2DM. Specifically small HDL particles played a role in cellular cholesterol efflux, and had antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic capacities [40,41]. These observations support our findings in DSA and we hypothesize that in DSA with T2DM, impaired HDL function might be explained by the loss of functional small HDL subfractions; while in DwC with T2DM, increased triglyceride content in small HDL subclass might lead to HDL dysfunction. Our in-house HDL functional assay revealed that individuals with T2DM in both ethnic groups had significantly reduced anti-thrombotic capacity due to impaired HDL function, which is also consistent with earlier findings showing that individuals with T2DM had impaired HDL function in terms of its capacity to suppress TNF-induced vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells *in vitro* [19]. A previous study by Bakker *et al.* reported that in obese DSA, without T2DM, only the ability of HDL to prevent LDL oxidation was reduced in overweight DSA when compared to obese DwC (without T2DM) [25]. Following these findings, we discovered that obese DSA with T2DM had a specific change in HDL composition, implying that T2DM could be an additional ethnicity-specific risk factor for cardiovascular disease.

HDL-4 subfractions in DSA with T2DM had the most clinical relevance, but not in DwC with T2DM. There were notable associations between HDL-4 subfractions with both waist circumference and waist-to-hip ratio, which were reported in the HELIUS study as critical predictors for T2DM regardless of ethnic background [42]. Additionally, lipid content of HDL-

4 was discovered to have associations with glycemic control parameters, which were risk factors for diabetes progression [43-45]. In the current study, we also observed that HDL-4 subfractions negatively correlated with diabetes duration in DSA, and exhibited remarkable differences between patients with and without pan-microvascular complications, particularly neuropathy and retinopathy. In contrast, there was no link between HDL compositional differences and diabetes-related complications in DwC subjects with T2DM. This could indicate that changes in HDL composition occur as a consequence of hyperglycemia and disease duration rather than as a driving factor associated with diabetes-related complications. HDL-related pharmacological strategies that have been tested involving HDL-mimetic peptides, showed remarkable effects on reducing inflammation, preventing oxidation, and promoting cholesterol efflux [46-48]. We found that HDL-4 subfractions were associated with disease duration in DSA with T2DM, suggesting the necessity of future studies to test the HDL-mimetic peptide effects on T2DM in individuals of SA descent.

The strength of our study is that we measured the HDL composition in detail in two ethnic groups of diabetic and non-diabetic individuals. Our findings further indicated impaired HDL function in T2DM, linking it with diabetes-related complications. Meanwhile, we revealed ethnic differences in HDL composition. Of note, there are still several limitations to our study. First, our study is a cross-sectional study, which precludes statements on causality. Second, sample sizes are relatively small, which might limit generalization potential and preclude stratification analyses. Third, Dutch South Asians with T2DM have longer disease duration than Dutch white Caucasians with T2DM in our study, which may affect incidence of complications. However, DSA-T2DM showed much faster disease progression than DwC-T2DM, which is a typical ethnic hallmark; future studies with larger sample size and multiple ethnicities are needed to verify our observations. Fourth, statin use could also affect HDL concentration, composition, and function [49,50], suggesting that differences in HDL composition between healthy controls and T2DM may not be solely due to diabetes status. Fifth, due to limited plasma availability, we were only able to perform an in-house HDL anti-

thrombotic capacity assay, without performing cholesterol efflux capacity, anti-oxidation capacity, or anti-inflammatory capacity measurements. Besides, we could not perform experiments to evaluate the anti-thrombotic capacity between large/buoyant HDL and small dense HDL. Sixth, the biological response of HUVECs is variable; therefore, our in-house assay is not comparable to clinical chemistry determinations.

Conclusions

In conclusion, Dutch T2DM patients of both white Caucasian and South Asian descent exhibited altered HDL composition when compared to healthy individuals, but revealed a distinct phenotype. In Dutch South Asian subjects, lower ApoA2 and HDL-4 were associated with higher incidence of diabetes-related complications such as retinopathy and neuropathy, suggesting that they could be used as ethnicity-specific biomarkers for T2DM patients.

Abbreviations

ApoA1: apolipoprotein A1; ApoA2: apolipoprotein A2; ACE: angiotensin-converting enzyme; B.I.LISA: Bruker IVDr Lipoprotein Subclass Analysis; BMI: body mass index; BSA: body surface area; CI: confidence interval; DSA: Dutch South Asian; DwC: Dutch white Caucasian; ETP: endogenous thrombin potential; GGT: gamma-glutamyl transferase; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; HUVEC: human umbilical vein endothelial cell; LDL: low-density lipoprotein; MAGNA VICTORIA: MAGNetic resonance Assessment of VICTOza efficacy in the Regression of cardiovascular dysfunction In type 2 diAbetes mellitus; MLR: multinomial logistic regression analysis; NMR: Nuclear magnetic resonance; OR: odds ratio; RCT: randomized controlled trial; SA: South Asian; SAT: subcutaneous adipose tissue; SD: standard deviation; T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus; TBF: total body fat percentage; TNF-α: tumor necrosis factor α; VAT: visceral adipose tissue; WC: white Caucasian.

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Authors' contributions

LY, MAG, and BMvdB contributed to the study concept, design, and analysis; LY analysed and interpreted data, and critically revised the manuscript; RL-G interpreted data and revised the manuscript; AV performed NMR measurements; HJvE and MBB collected data for the MAGNA VICTORIA studies, IMJ supervised the MAGNA VICTORIA studies, with HJL serving as study director; PCNR and MAG interpreted NMR data; and LY, TJR, and BMvdB drafted the manuscript. The final manuscript was read, commented on, and approved by all authors.

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Availability of data and materials

All data and methods supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The T2DM participants were from baseline samples of two previous randomized controlled trials (ClinicalTrials. gov NCT01761318 and NCT02660047). The study protocol was approved by the Institutional Review Board (Leiden University Medical Center, Leiden, The Netherlands), and all participants provided written informed consent.

Consent for publication

The manuscript was approved by all authors for publication.

Competing interests

The authors declare that they have no competing interests.

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Supporting information

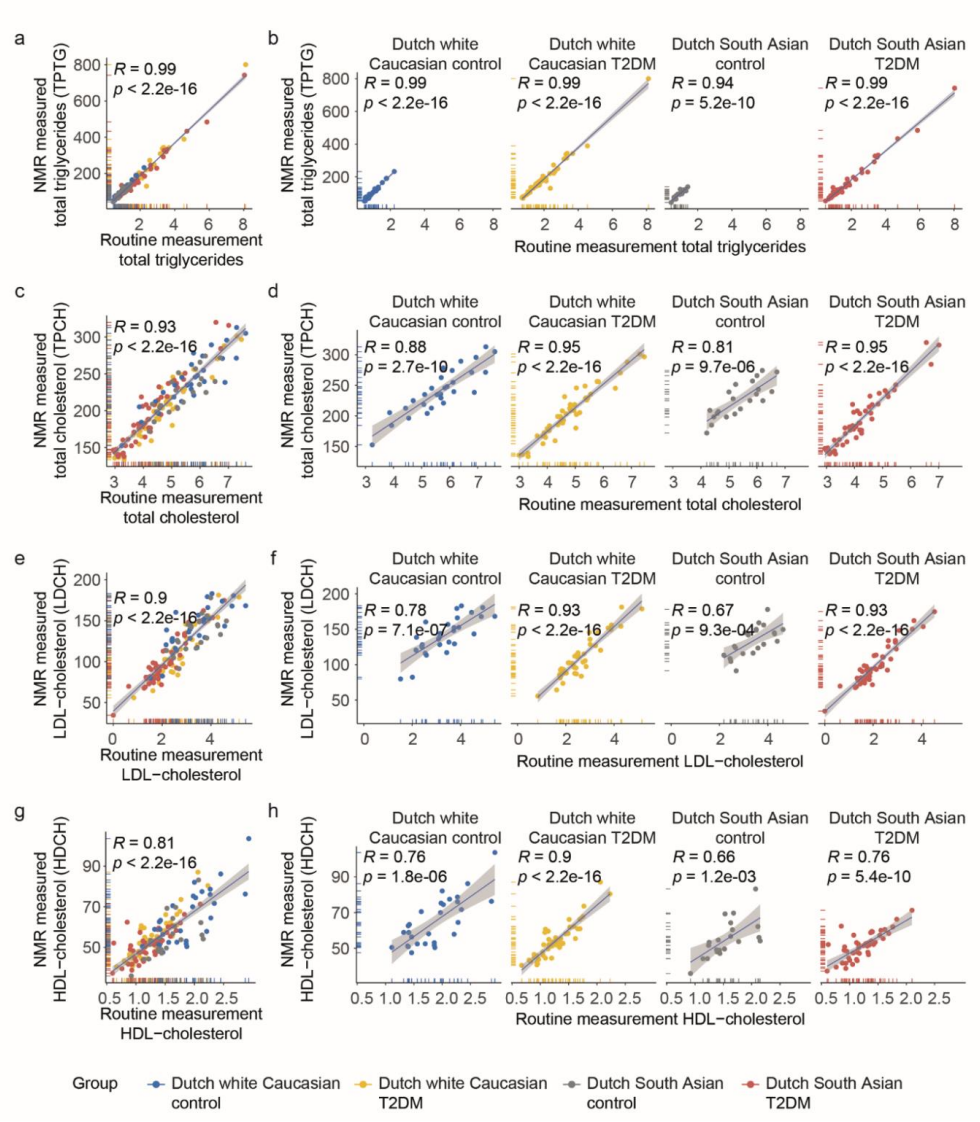

Figure S1. Association between lipids and apolipoproteins measured by NMR spectroscopy vs clinical chemistry approach. Pearson's correlation between routine total triglyceride levels (mmol/L) and NMR extracted total triglyceride concentrations (TPTG, mg/dL) in (a) the total cohort and (b) the individual groups. Pearson's correlation between routine total cholesterol (mmol/L) levels and NMR extracted total cholesterol (TPCH, mg/dL) concentrations in (c) the total cohort and (d) the individual groups. Pearson's

correlation between routine LDL-cholesterol (mmol/L) levels and NMR extracted LDL-cholesterol (LDCH, mg/dL) concentrations in (e) the total cohort and (f) the individual groups. Pearson's correlation between routine HDL-cholesterol levels (mmol/L) and NMR extracted HDL-cholesterol concentrations (mg/dL) in (g) the total cohort and (h) the individual groups.

Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.

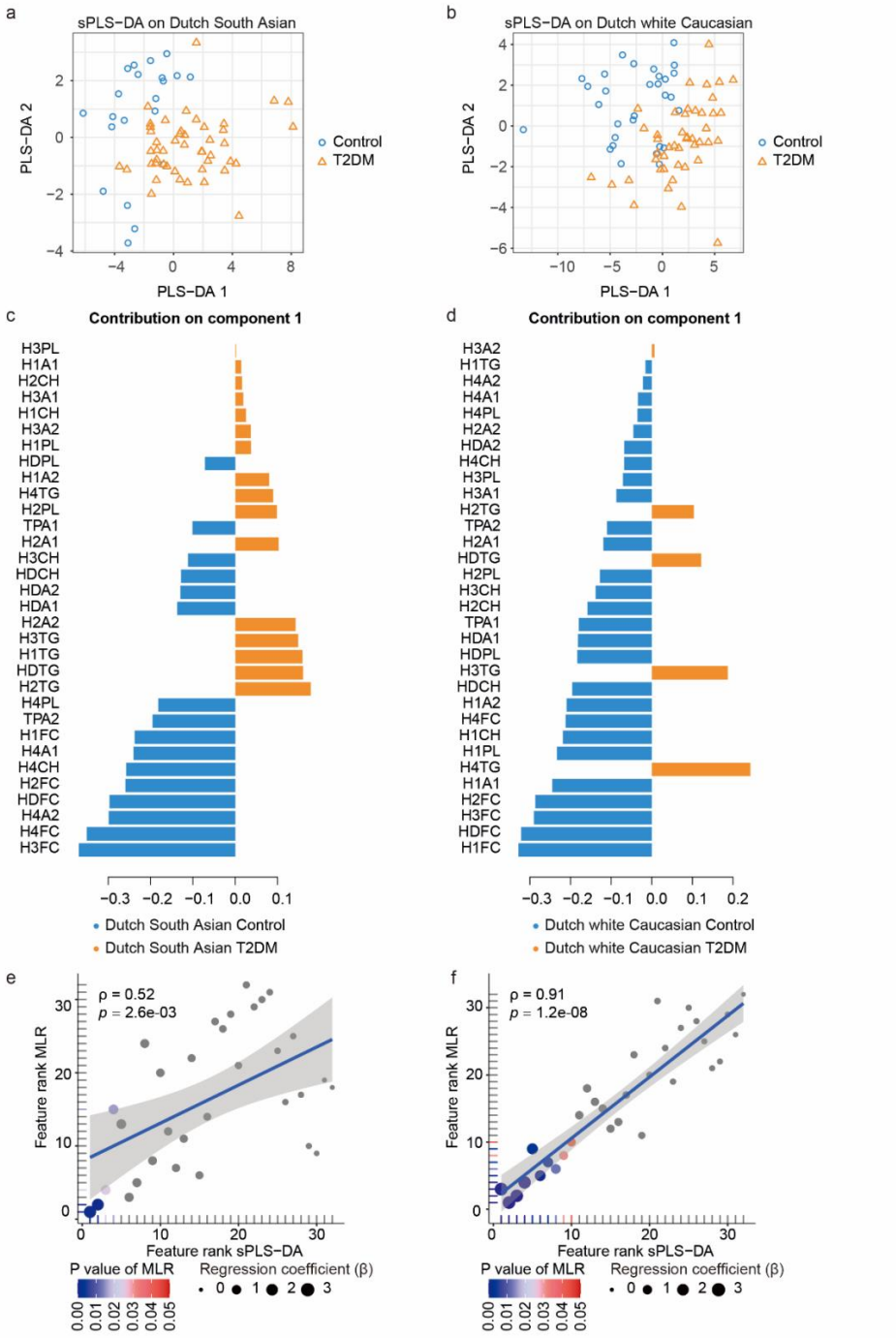
a
sPLS−DA on Dutch South Asian
PLS−DA 2
PLS−DA 1
Control
T2DM
b
sPLS−DA on Dutch white Caucasian
PLS−DA 2
PLS−DA 1
Control
T2DM
c
Contribution on component 1
H3PL
H1A1
H2CH
H3A1
H1CH
H3A2
H1PL
HDPL
H1A2
H4TG
H2PL
TPA1
H2A1
H3CH
HDCH
HDA2
HDA1
H2A2
H3TG
H1TG
HDTG
H2TG
H4PL
TPA2
H1FC
H4A1
H4CH
H2FC
HDFC
H4A2
H4FC
H3FC
Dutch South Asian Control
Dutch South Asian T2DM
d
Contribution on component 1
H3A2
H1TG
H4A2
H4A1
H4PL
H2A2
HDA2
H4CH
H3PL
H3A1
H2TG
TPA2
H2A1
HDTG
H2PL
H3CH
H2CH
TPA1
HDA1
HDPL
H3TG
HDCH
H1A2
H4FC
H1CH
H1PL
H4TG
H1A1
H2FC
H3FC
HDFC
H1FC
Dutch white Caucasian Control
Dutch white Caucasian T2DM
e
ρ = 0.52
p = 2.6e-03
Feature rank MLR
Feature rank sPLS−DA
P value of MLR
Regression coefficient (β)
f
ρ = 0.91
p = 1.2e-08
Feature rank MLR
Feature rank sPLS−DA
P value of MLR
Regression coefficient (β)

Figure S2. Sparse partial least squares discriminant analysis (sPLS-DA) differentiates patients with T2DM from healthy controls and T2DM in two ethnicities. sPLS-DA performed on HDL-related features separates patients with T2DM from healthy controls in (a) Dutch South Asians and (b) Dutch white Caucasians. Loading values of features in (c) Dutch South Asians and (d) Dutch white Caucasians with the contribution to differentiating T2DM from healthy control using sPLS-DA. Spearman's correlation between the rank generated from multinomial logistic regression (MLR) and sPLS-DA in (e) Dutch South Asians and (f) Dutch white Caucasians. Dot color represents the p-value of MLR, and dot size represents the absolute value of regression coefficient.

Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.

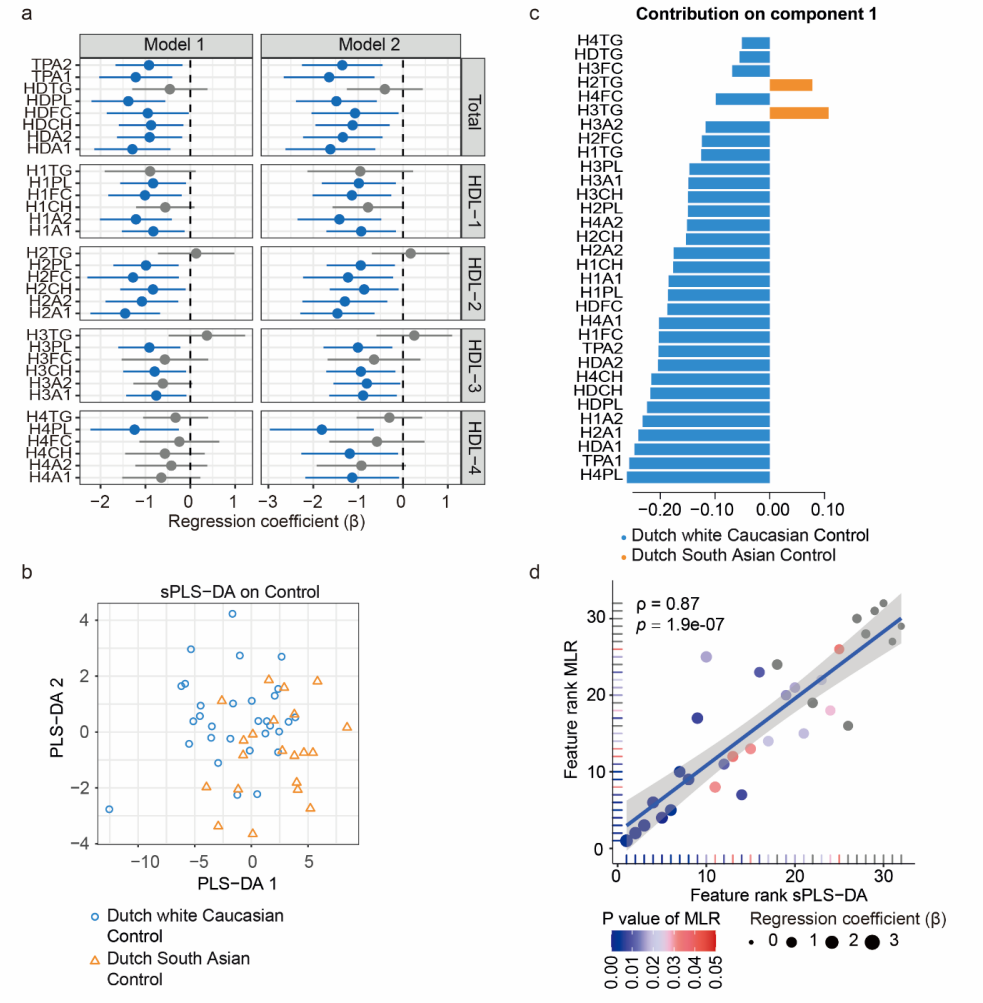

Figure S3. Different HDL composition between health individuals of Dutch South Asian and Dutch white Caucasian. (a) Forest plot of differential lipoprotein subfractions between health individuals of Dutch South Asian and Dutch white Caucasian. Regression coefficients and 95% confidence intervals are depicted by horizontal lines with dots. Non-significant associations are represented in grey, and substantial negative associations are represented in blue. (b) sPLS-DA performed on HDL-related features separates healthy individuals of Dutch South Asians from those of Dutch white Caucasians. (c) Loading values of features with the contribution to differentiating ealthy individuals of Dutch South Asians from those of Dutch white Caucasians using sPLS-DA. (d) Spearman's correlation between the rank generated from multinomial logistic regression (MLR) and sPLS-DA. Dot colors represent p-values of MLR, and dot sizes represent the absolute values of regression coefficients.

Abbreviations: *A1*: apolipoprotein A1; *A2*: apolipoprotein A2; *CH*: cholesterol; *FC*: free cholesterol; *PL*: phospholipid; *TG*: triglyceride. Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.

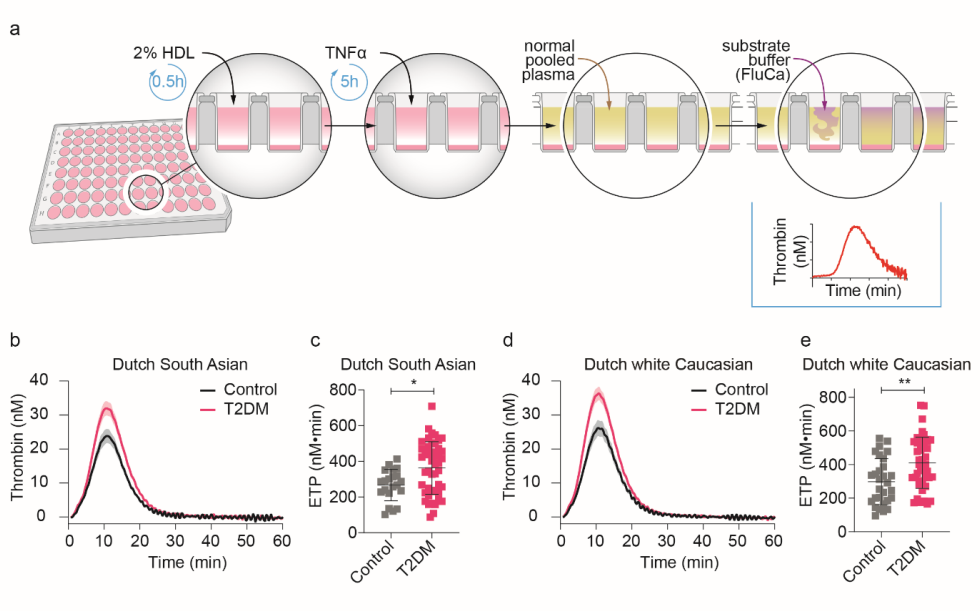

Figure S4. Impaired HDL anti-thrombotic capacity in T2DM in both ethnicities. (a) Scheme of HDL anti-thrombotic capacity assay. (b) Anti-thrombotic capacity between healthy and diabetic indivuduals in Dutch South Asian visualzied by thrombin generation curve. (c) Difference of endogenous thrombin potential (ETP) between T2DM and control in Dutch South Asians. (d) Anti-thrombotic capacity measurement between healthy and diabetic indivuduals in Dutch white Caucasian visualzied by thrombin generation curve. (e) Difference of ETP between T2DM and control in Dutch white Caucasians. Graphs represent means ± SEM for the thrombin generation curve and means ± SD for the dot plots. Non-paired two-tailed Mann-Whitney-Wilcoxon tests were performed; *p<0.05, **p<0.01.

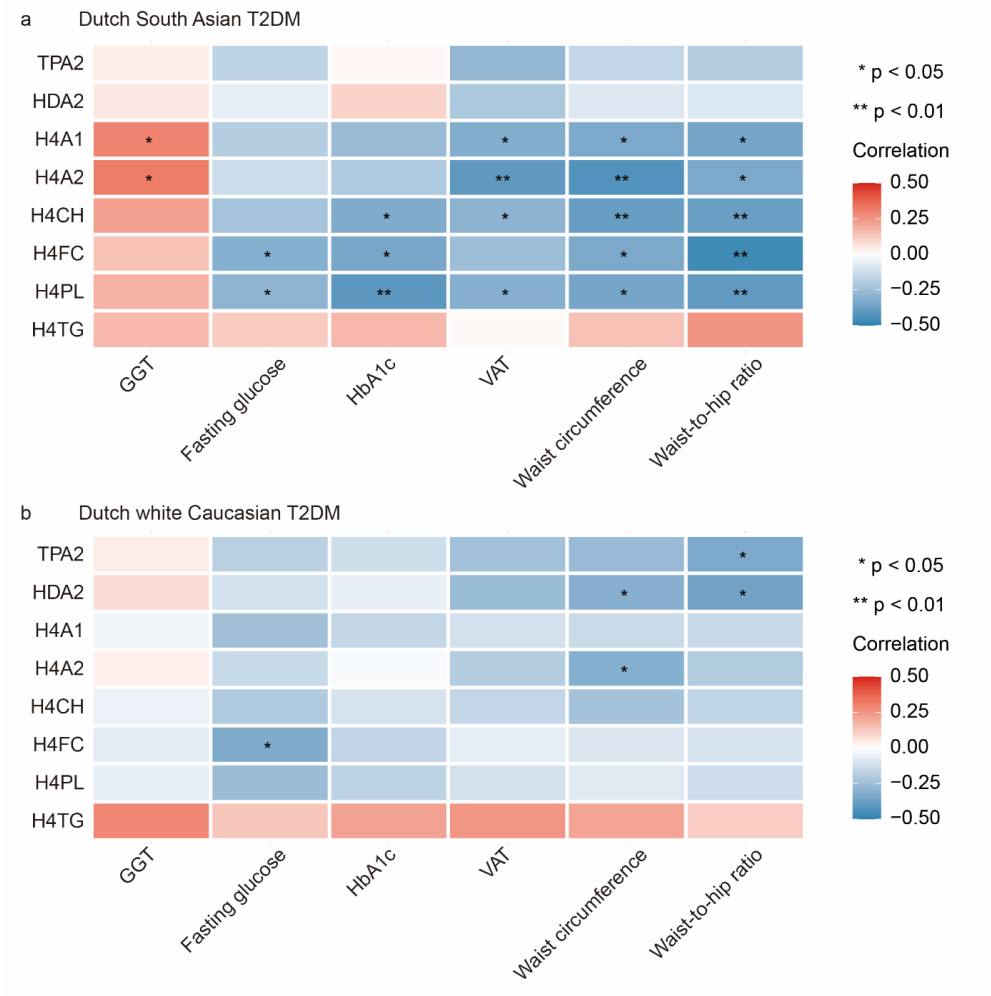

Figure S5. Correlation between differential HDL composition and clinical outcomes. Pearson's Correlation between the levels of VAT, waist circumference, waist-to-hip ratio, glucose, HbA1c, and GGT with differential HDL composition in (a) Dutch South Asians and (b) Dutch white Caucasians. Pearson's correlation analysis was performed. The color of scale bar represented Pearson's correlation R. *p<0.05; **p<0.01.

Abbreviations: *GGT*: gamma-glutamyl transferase; *HbA1c*: hemoglobin A1c; *VAT*: visceral adipose tissue. Abbreviations of lipoprotein main fractions and subfractions are shown in Supplemental Table S1.

Table S1: Quantified HDL lipoprotein main fractions and subfractions.

Abbreviation	Lipoprotein subfraction
TPA1	Total apolipoprotein A1 (ApoA1)
TPA2	Total apolipoprotein A2 (ApoA2)
HDA1	ApoA1 content in total HDL
HDA2	ApoA2 content in total HDL
HDCH	Cholesterol content in total HDL
HDFC	Free Cholesterol content in total HDL
HDPL	Phospholipid content in total HDL
HDTG	Triglyceride content in total HDL
H1A1	ApoA1 content in HDL-1 subclass
H1A2	ApoA2 content in HDL-1 subclass
H1CH	Cholesterol content in HDL-1 subclass
H1FC	Free Cholesterol content HDL-1 subclass
H1PL	Phospholipid content in HDL-1 subclass
H1TG	Triglyceride content in HDL-1 subclass
H2A1	ApoA1 content in HDL-2 subclass
H2A2	ApoA2 content in HDL-2 subclass
H2CH	Cholesterol content in HDL-2 subclass
H2FC	Free Cholesterol content HDL-2 subclass
H2PL	Phospholipid content in HDL-2 subclass
H2TG	Triglyceride content in HDL-2 subclass
H3A1	ApoA1 content in HDL-3 subclass
H3A2	ApoA2 content in HDL-3 subclass
H3CH	Cholesterol content in HDL-3 subclass
H3FC	Free Cholesterol content HDL-3 subclass
H3PL	Phospholipid content in HDL-3 subclass
H3TG	Triglyceride content in HDL-3 subclass

H4A1	ApoA1 content in HDL-4 subclass
H4A2	ApoA2 content in HDL-4 subclass
H4CH	Cholesterol content in HDL-4 subclass
H4FC	Free Cholesterol content HDL-4 subclass
H4PL	Phospholipid content in HDL-4 subclass
H4TG	Triglyceride content in HDL-4 subclass

Table S2. Differential HDL composition between DSA-T2DM and DSA-C based on multinomial logistic regression analysis. (Reference: healthy controls DSA-C)

Model 1: adjusted for age, gender, current smoking status

HDL composition	Regression coefficient (β)			Odds ratio (OR)			P-value
	β	CI2.5	CI97.5	OR	CI2.5	CI97.5	
HDCH	-0.70	-1.41	0.01	0.50	0.25	1.01	5.35E-02
TPA1	-0.36	-1.04	0.33	0.70	0.35	1.40	3.11E-01
TPA2	-0.69	-1.36	-0.02	0.50	0.26	0.98	4.33E-02
HDTG	1.00	0.25	1.75	2.72	1.28	5.76	9.19E-03
HDFC	-1.45	-2.29	-0.62	0.23	0.10	0.54	6.63E-04
HDPL	-0.28	-0.99	0.42	0.75	0.37	1.53	4.32E-01
HDA1	-0.49	-1.19	0.21	0.61	0.31	1.23	1.67E-01
HDA2	-0.41	-1.05	0.22	0.66	0.35	1.25	2.02E-01
H1TG	1.21	0.23	2.18	3.34	1.26	8.83	1.49E-02
H2TG	0.97	0.27	1.67	2.63	1.31	5.29	6.72E-03
H3TG	0.85	0.19	1.51	2.34	1.21	4.51	1.12E-02
H4TG	0.45	-0.19	1.09	1.57	0.83	2.97	1.66E-01
H1CH	0.00	-0.62	0.63	1.00	0.54	1.88	9.92E-01
H2CH	0.11	-0.55	0.78	1.12	0.57	2.18	7.39E-01
H3CH	-0.31	-0.94	0.31	0.73	0.39	1.37	3.28E-01
H4CH	-1.25	-2.09	-0.42	0.29	0.12	0.66	3.39E-03
H1FC	-1.26	-2.07	-0.46	0.28	0.13	0.63	2.14E-03
H2FC	-1.07	-1.83	-0.30	0.34	0.16	0.74	6.30E-03
H3FC	-1.98	-2.93	-1.04	0.14	0.05	0.35	3.76E-05
H4FC	-2.06	-3.00	-1.12	0.13	0.05	0.33	1.69E-05
H1PL	0.12	-0.57	0.82	1.13	0.56	2.26	7.31E-01
H2PL	0.50	-0.14	1.14	1.65	0.87	3.14	1.24E-01
H3PL	0.13	-0.45	0.71	1.14	0.64	2.03	6.69E-01
H4PL	-0.76	-1.59	0.07	0.47	0.20	1.07	7.20E-02
H1A1	0.01	-0.64	0.67	1.01	0.53	1.95	9.70E-01
H2A1	0.47	-0.18	1.12	1.60	0.84	3.06	1.54E-01
H3A1	0.20	-0.39	0.79	1.22	0.68	2.21	5.04E-01
H4A1	-1.07	-1.90	-0.24	0.34	0.15	0.79	1.17E-02
H1A2	0.50	-0.24	1.25	1.66	0.79	3.48	1.84E-01
H2A2	0.98	0.21	1.76	2.67	1.23	5.79	1.26E-02
H3A2	0.28	-0.31	0.87	1.32	0.73	2.38	3.58E-01
H4A2	-1.41	-2.24	-0.59	0.24	0.11	0.56	8.18E-04

Model 2: Model 1 + BMI

HDL composition	Regression coefficient (β)			Odds ratio (OR)			P-value
	β	CI2.5	CI97.5	OR	CI2.5	CI97.5	
HDCH	-0.03	-0.88	0.83	0.97	0.41	2.29	9.52E-01
TPA1	0.38	-0.58	1.35	1.47	0.56	3.85	4.36E-01
TPA2	-0.08	-0.96	0.80	0.92	0.38	2.22	8.52E-01
HDTG	0.67	-0.21	1.55	1.96	0.81	4.72	1.36E-01
HDFC	-1.18	-2.20	-0.15	0.31	0.11	0.86	2.44E-02
HDPL	0.19	-0.69	1.07	1.21	0.50	2.92	6.66E-01
HDA1	0.20	-0.75	1.14	1.22	0.47	3.13	6.84E-01
HDA2	0.17	-0.69	1.03	1.19	0.50	2.81	6.99E-01
H1TG	1.14	-0.06	2.35	3.14	0.94	10.44	6.25E-02
H2TG	0.60	-0.23	1.44	1.83	0.80	4.20	1.55E-01
H3TG	0.52	-0.27	1.32	1.69	0.76	3.74	1.97E-01
H4TG	0.21	-0.60	1.02	1.23	0.55	2.76	6.11E-01
H1CH	0.39	-0.45	1.22	1.47	0.64	3.40	3.67E-01
H2CH	0.34	-0.47	1.14	1.40	0.62	3.14	4.14E-01
H3CH	0.04	-0.75	0.82	1.04	0.47	2.27	9.25E-01
H4CH	-0.57	-1.62	0.49	0.57	0.20	1.63	2.92E-01
H1FC	-0.82	-1.79	0.16	0.44	0.17	1.17	1.00E-01
H2FC	-0.87	-1.84	0.10	0.42	0.16	1.10	7.84E-02
H3FC	-2.32	-3.72	-0.92	0.10	0.02	0.40	1.13E-03
H4FC	-2.12	-3.38	-0.85	0.12	0.03	0.43	1.08E-03
H1PL	0.44	-0.44	1.32	1.55	0.64	3.73	3.31E-01
H2PL	0.57	-0.19	1.34	1.78	0.83	3.81	1.41E-01
H3PL	0.35	-0.40	1.10	1.42	0.67	3.01	3.63E-01
H4PL	-0.15	-1.12	0.81	0.86	0.33	2.26	7.57E-01
H1A1	0.25	-0.60	1.11	1.29	0.55	3.03	5.64E-01
H2A1	0.72	-0.06	1.49	2.05	0.94	4.45	7.13E-02
H3A1	0.34	-0.40	1.09	1.41	0.67	2.98	3.66E-01
H4A1	-0.63	-1.71	0.45	0.53	0.18	1.57	2.52E-01
H1A2	0.86	-0.09	1.81	2.35	0.91	6.08	7.70E-02
H2A2	1.17	0.22	2.11	3.21	1.24	8.28	1.59E-02
H3A2	0.45	-0.29	1.19	1.57	0.75	3.30	2.30E-01
H4A2	-0.95	-2.05	0.16	0.39	0.13	1.17	9.25E-02

Table S3. Differential HDL composition between DwC-T2DM and DwC-C based on multinomial logistic regression analysis. (Reference: DwC-C)

Model 1: adjusted for age, gender, current smoking status

HDL composition	Regression coefficient (β)			Odds ratio (OR)			P-value
	β	CI2.5	CI97.5	OR	CI2.5	CI97.5	
HDCH	-0.99	-1.59	-0.38	0.37	0.20	0.68	1.40E-03
TPA1	-0.97	-1.60	-0.33	0.38	0.20	0.72	3.03E-03
TPA2	-0.44	-0.97	0.09	0.65	0.38	1.10	1.07E-01
HDTG	0.60	0.00	1.20	1.82	1.00	3.31	4.90E-02
HDFC	-2.72	-3.70	-1.74	0.07	0.02	0.18	5.86E-08
HDPL	-1.05	-1.68	-0.42	0.35	0.19	0.66	1.14E-03
HDA1	-1.06	-1.74	-0.38	0.35	0.18	0.68	2.12E-03
HDA2	-0.21	-0.71	0.29	0.81	0.49	1.33	4.06E-01
H1TG	-0.06	-0.62	0.50	0.95	0.54	1.66	8.44E-01
H2TG	0.73	0.02	1.44	2.07	1.02	4.20	4.29E-02
H3TG	1.23	0.47	1.99	3.42	1.60	7.31	1.47E-03
H4TG	1.48	0.78	2.17	4.39	2.19	8.79	3.11E-05
H1CH	-1.19	-1.84	-0.54	0.30	0.16	0.58	3.10E-04
H2CH	-0.81	-1.39	-0.24	0.44	0.25	0.79	5.70E-03
H3CH	-0.67	-1.22	-0.13	0.51	0.30	0.88	1.59E-02
H4CH	-0.37	-0.99	0.25	0.69	0.37	1.28	2.42E-01
H1FC	-2.91	-3.94	-1.89	0.05	0.02	0.15	2.70E-08
H2FC	-2.63	-3.64	-1.62	0.07	0.03	0.20	3.31E-07
H3FC	-2.35	-3.31	-1.39	0.10	0.04	0.25	1.56E-06
H4FC	-1.44	-2.23	-0.66	0.24	0.11	0.52	3.21E-04
H1PL	-1.52	-2.24	-0.80	0.22	0.11	0.45	3.34E-05
H2PL	-0.69	-1.26	-0.12	0.50	0.28	0.89	1.82E-02
H3PL	-0.35	-0.89	0.19	0.70	0.41	1.21	2.01E-01
H4PL	-0.20	-0.85	0.45	0.82	0.43	1.56	5.42E-01
H1A1	-1.69	-2.44	-0.95	0.18	0.09	0.39	8.99E-06
H2A1	-0.58	-1.15	-0.01	0.56	0.32	0.99	4.43E-02
H3A1	-0.34	-0.86	0.17	0.71	0.43	1.18	1.89E-01
H4A1	-0.14	-0.71	0.42	0.87	0.49	1.52	6.16E-01
H1A2	-1.00	-1.60	-0.40	0.37	0.20	0.67	1.09E-03
H2A2	-0.13	-0.59	0.34	0.88	0.55	1.41	6.00E-01
H3A2	0.08	-0.39	0.56	1.09	0.68	1.75	7.30E-01
H4A2	-0.07	-0.60	0.47	0.94	0.55	1.59	8.09E-01

Model 2: Model 1 + BMI

HDL composition	Regression coefficient (β)			Odds ratio (OR)			P-value
	β	CI2.5	CI97.5	OR	CI2.5	CI97.5	
HDCH	-0.37	-1.11	0.37	0.69	0.33	1.45	3.29E-01
TPA1	-0.63	-1.38	0.12	0.53	0.25	1.12	9.76E-02
TPA2	-0.14	-0.87	0.58	0.87	0.42	1.79	7.01E-01
HDTG	0.18	-0.58	0.94	1.20	0.56	2.57	6.43E-01
HDFC	-2.57	-3.75	-1.39	0.08	0.02	0.25	1.91E-05
HDPL	-0.58	-1.39	0.22	0.56	0.25	1.25	1.56E-01
HDA1	-0.68	-1.47	0.10	0.50	0.23	1.10	8.72E-02
HDA2	0.07	-0.65	0.79	1.07	0.52	2.20	8.52E-01
H1TG	-0.23	-0.97	0.51	0.79	0.38	1.67	5.42E-01
H2TG	0.14	-0.77	1.04	1.15	0.46	2.84	7.70E-01
H3TG	0.59	-0.30	1.48	1.80	0.74	4.41	1.95E-01
H4TG	1.17	0.25	2.09	3.21	1.28	8.08	1.31E-02
H1CH	-0.85	-1.63	-0.08	0.43	0.20	0.92	3.09E-02
H2CH	-0.64	-1.42	0.14	0.53	0.24	1.15	1.06E-01
H3CH	-0.43	-1.13	0.28	0.65	0.32	1.32	2.33E-01
H4CH	-0.14	-0.97	0.69	0.87	0.38	1.99	7.35E-01
H1FC	-2.60	-3.81	-1.40	0.07	0.02	0.25	2.38E-05
H2FC	-2.45	-3.61	-1.28	0.09	0.03	0.28	3.81E-05
H3FC	-2.73	-4.13	-1.32	0.07	0.02	0.27	1.45E-04
H4FC	-1.72	-2.84	-0.59	0.18	0.06	0.55	2.81E-03
H1PL	-1.33	-2.24	-0.43	0.26	0.11	0.65	3.95E-03
H2PL	-0.65	-1.42	0.12	0.52	0.24	1.12	9.67E-02
H3PL	-0.23	-0.95	0.49	0.80	0.39	1.64	5.38E-01
H4PL	-0.16	-1.08	0.75	0.85	0.34	2.13	7.30E-01
H1A1	-1.63	-2.59	-0.67	0.20	0.07	0.51	8.73E-04
H2A1	-0.24	-0.98	0.51	0.79	0.37	1.66	5.29E-01
H3A1	-0.37	-1.03	0.30	0.69	0.36	1.34	2.76E-01
H4A1	-0.13	-0.94	0.68	0.88	0.39	1.97	7.49E-01
H1A2	-0.76	-1.49	-0.03	0.47	0.23	0.97	4.13E-02
H2A2	-0.18	-0.77	0.42	0.84	0.46	1.51	5.60E-01
H3A2	0.02	-0.61	0.65	1.02	0.54	1.91	9.52E-01
H4A2	0.17	-0.69	1.03	1.19	0.50	2.79	6.98E-01

Table S4. Differential HDL composition between DSA-T2DM and DwC-T2DM based on multinomial logistic regression analysis. (Reference: DwC-T2DM)

Model 1: adjusted for age, gender, current smoking status

HDL composition	Regression coefficient (β)			P-value
	β	CI2.5	CI97.5	
HDCH	-0.58	-1.18	0.01	5.37E-02
TPA1	-0.61	-1.15	-0.06	2.88E-02
TPA2	-1.17	-1.75	-0.60	6.77E-05
HDTG	-0.06	-0.46	0.35	7.84E-01
HDFC	0.32	-0.27	0.91	2.93E-01
HDPL	-0.61	-1.19	-0.03	3.80E-02
HDA1	-0.72	-1.29	-0.15	1.31E-02
HDA2	-1.11	-1.66	-0.56	8.19E-05
H1TG	0.37	-0.11	0.85	1.32E-01
H2TG	0.37	-0.10	0.83	1.19E-01
H3TG	-0.01	-0.43	0.41	9.57E-01
H4TG	-1.36	-1.98	-0.73	2.03E-05
H1CH	0.64	0.03	1.25	4.06E-02
H2CH	0.09	-0.44	0.62	7.31E-01
H3CH	-0.44	-0.94	0.06	8.79E-02
H4CH	-1.45	-2.12	-0.77	2.59E-05
H1FC	0.64	-0.06	1.35	7.37E-02
H2FC	0.28	-0.26	0.83	3.09E-01
H3FC	-0.20	-0.72	0.32	4.46E-01
H4FC	-0.86	-1.45	-0.27	4.22E-03
H1PL	0.82	0.16	1.48	1.55E-02
H2PL	0.21	-0.31	0.72	4.32E-01
H3PL	-0.43	-0.92	0.05	7.91E-02
H4PL	-1.80	-2.60	-1.00	9.58E-06
H1A1	0.88	0.20	1.56	1.09E-02
H2A1	-0.40	-0.93	0.13	1.37E-01
H3A1	-0.21	-0.69	0.26	3.75E-01
H4A1	-1.57	-2.25	-0.89	6.14E-06
H1A2	0.29	-0.28	0.86	3.19E-01
H2A2	0.03	-0.42	0.48	8.95E-01
H3A2	-0.42	-0.88	0.05	7.78E-02
H4A2	-1.77	-2.47	-1.07	8.22E-07

Model 2: Model 1 + BMI

HDL composition	Regression coefficient (β)			P-value
	β	CI2.5	CI97.5	
HDCH	-0.78	-1.44	-0.12	1.99E-02
TPA1	-0.63	-1.20	-0.05	3.18E-02
TPA2	-1.30	-1.96	-0.63	1.46E-04
HDTG	0.09	-0.37	0.54	7.12E-01
HDFC	0.33	-0.33	0.98	3.29E-01
HDPL	-0.71	-1.37	-0.04	3.64E-02
HDA1	-0.74	-1.34	-0.13	1.67E-02
HDA2	-1.24	-1.89	-0.59	1.77E-04
H1TG	0.42	-0.10	0.94	1.13E-01
H2TG	0.64	0.06	1.22	3.17E-02
H3TG	0.19	-0.30	0.67	4.59E-01
H4TG	-1.26	-1.93	-0.59	2.26E-04
H1CH	0.46	-0.20	1.11	1.69E-01
H2CH	0.11	-0.52	0.74	7.24E-01
H3CH	-0.47	-1.03	0.09	9.76E-02
H4CH	-1.61	-2.38	-0.84	4.58E-05
H1FC	0.65	-0.16	1.46	1.15E-01
H2FC	0.35	-0.27	0.98	2.65E-01
H3FC	-0.24	-0.79	0.31	3.96E-01
H4FC	-0.98	-1.64	-0.32	3.83E-03
H1PL	0.78	0.02	1.54	4.38E-02
H2PL	0.28	-0.32	0.89	3.57E-01
H3PL	-0.43	-0.97	0.11	1.19E-01
H4PL	-1.80	-2.68	-0.93	5.52E-05
H1A1	0.95	0.16	1.74	1.78E-02
H2A1	-0.51	-1.12	0.10	1.04E-01
H3A1	-0.18	-0.68	0.33	4.93E-01
H4A1	-1.63	-2.41	-0.85	4.10E-05
H1A2	0.20	-0.44	0.83	5.41E-01
H2A2	0.05	-0.43	0.52	8.45E-01
H3A2	-0.37	-0.86	0.12	1.38E-01
H4A2	-2.05	-2.93	-1.17	4.92E-06

Model 3: Model 2 + Diabetes duration

HDL composition	Regression coefficient (β)			P-value
	β	CI2.5	CI97.5	
HDCH	-0.41	-1.07	0.24	2.18E-01
TPA1	-0.36	-0.92	0.20	2.05E-01
TPA2	-0.93	-1.63	-0.23	9.07E-03
HDTG	0.12	-0.38	0.63	6.35E-01
HDFC	0.37	-0.35	1.09	3.12E-01
HDPL	-0.44	-1.16	0.28	2.32E-01
HDA1	-0.42	-1.01	0.17	1.64E-01
HDA2	-0.91	-1.59	-0.23	8.36E-03
H1TG	0.45	-0.15	1.06	1.41E-01
H2TG	0.63	-0.03	1.29	5.98E-02
H3TG	0.18	-0.35	0.71	5.02E-01
H4TG	-1.34	-2.18	-0.50	1.76E-03
H1CH	0.46	-0.26	1.17	2.09E-01
H2CH	0.30	-0.42	1.03	4.14E-01
H3CH	-0.31	-0.90	0.29	3.13E-01
H4CH	-1.13	-1.88	-0.38	3.13E-03
H1FC	0.58	-0.25	1.40	1.71E-01
H2FC	0.46	-0.26	1.18	2.10E-01
H3FC	-0.15	-0.79	0.48	6.38E-01
H4FC	-0.75	-1.43	-0.06	3.36E-02
H1PL	0.82	-0.05	1.70	6.48E-02
H2PL	0.42	-0.30	1.14	2.54E-01
H3PL	-0.36	-0.97	0.26	2.56E-01
H4PL	-1.22	-2.02	-0.42	2.92E-03
H1A1	1.12	0.21	2.04	1.60E-02
H2A1	-0.31	-0.98	0.36	3.60E-01
H3A1	-0.14	-0.68	0.39	5.99E-01
H4A1	-1.14	-1.90	-0.38	3.25E-03
H1A2	0.26	-0.43	0.95	4.61E-01
H2A2	0.02	-0.50	0.54	9.33E-01
H3A2	-0.31	-0.84	0.22	2.54E-01
H4A2	-1.78	-2.83	-0.73	9.27E-04

Table S5. Differential HDL composition between DSA-C and DwC-C based on multinomial logistic regression analysis. (Reference: DwC-C)

Model 1: adjusted for age, gender, current smoking status

HDL composition	Regression coefficient (β)			P-value
	β	CI2.5	CI97.5	
HDCH	-0.87	-1.60	-0.15	1.77E-02
TPA1	-1.22	-2.03	-0.40	3.42E-03
TPA2	-0.92	-1.67	-0.17	1.57E-02
HDTG	-0.45	-1.29	0.38	2.88E-01
HDFC	-0.95	-1.86	-0.03	4.19E-02
HDPL	-1.38	-2.20	-0.56	9.91E-04
HDA1	-1.29	-2.14	-0.44	2.90E-03
HDA2	-0.91	-1.63	-0.18	1.44E-02
H1TG	-0.90	-1.91	0.12	8.40E-02
H2TG	0.13	-0.72	0.98	7.62E-01
H3TG	0.37	-0.48	1.22	3.95E-01
H4TG	-0.33	-1.05	0.40	3.76E-01
H1CH	-0.56	-1.21	0.09	9.32E-02
H2CH	-0.83	-1.56	-0.10	2.53E-02
H3CH	-0.79	-1.50	-0.09	2.67E-02
H4CH	-0.57	-1.45	0.32	2.11E-01
H1FC	-1.01	-1.83	-0.19	1.59E-02
H2FC	-1.28	-2.29	-0.26	1.41E-02
H3FC	-0.57	-1.53	0.40	2.50E-01
H4FC	-0.24	-1.14	0.65	5.91E-01
H1PL	-0.83	-1.56	-0.10	2.65E-02
H2PL	-0.99	-1.71	-0.26	7.76E-03
H3PL	-0.91	-1.60	-0.22	9.55E-03
H4PL	-1.24	-2.23	-0.26	1.35E-02
H1A1	-0.82	-1.52	-0.13	2.08E-02
H2A1	-1.45	-2.23	-0.67	2.54E-04
H3A1	-0.76	-1.43	-0.09	2.72E-02
H4A1	-0.65	-1.52	0.22	1.46E-01
H1A2	-1.21	-2.01	-0.41	3.12E-03
H2A2	-1.08	-1.89	-0.27	9.32E-03
H3A2	-0.61	-1.27	0.04	6.78E-02
H4A2	-0.42	-1.22	0.38	3.01E-01

Model 2: Model 1 + BMI

HDL composition	Regression coefficient (β)			P-value
	β	CI2.5	CI97.5	
HDCH	-1.12	-1.95	-0.30	7.84E-03
TPA1	-1.65	-2.66	-0.63	1.43E-03
TPA2	-1.35	-2.25	-0.45	3.18E-03
HDTG	-0.40	-1.25	0.44	3.49E-01
HDFC	-1.07	-2.04	-0.10	3.07E-02
HDPL	-1.48	-2.39	-0.58	1.23E-03
HDA1	-1.62	-2.62	-0.62	1.52E-03
HDA2	-1.34	-2.23	-0.45	3.06E-03
H1TG	-0.95	-2.13	0.23	1.13E-01
H2TG	0.17	-0.69	1.03	6.98E-01
H3TG	0.25	-0.59	1.09	5.58E-01
H4TG	-0.30	-1.04	0.43	4.15E-01
H1CH	-0.78	-1.57	0.01	5.29E-02
H2CH	-0.87	-1.63	-0.10	2.70E-02
H3CH	-0.94	-1.71	-0.17	1.69E-02
H4CH	-1.19	-2.27	-0.11	3.08E-02
H1FC	-1.14	-2.02	-0.26	1.10E-02
H2FC	-1.23	-2.23	-0.22	1.65E-02
H3FC	-0.65	-1.68	0.39	2.21E-01
H4FC	-0.58	-1.64	0.48	2.84E-01
H1PL	-0.98	-1.81	-0.16	1.92E-02
H2PL	-0.94	-1.70	-0.18	1.52E-02
H3PL	-1.00	-1.77	-0.24	1.03E-02
H4PL	-1.81	-2.97	-0.65	2.23E-03
H1A1	-0.93	-1.71	-0.16	1.85E-02
H2A1	-1.46	-2.29	-0.63	5.63E-04
H3A1	-0.89	-1.64	-0.14	2.07E-02
H4A1	-1.13	-2.18	-0.09	3.38E-02
H1A2	-1.42	-2.35	-0.48	2.94E-03
H2A2	-1.30	-2.24	-0.35	7.31E-03
H3A2	-0.81	-1.55	-0.06	3.34E-02
H4A2	-0.93	-1.92	0.07	6.78E-02