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Obesity-associated metabolites in relation to type 2 diabetes risk: A prospective nested case-control study of the CARRS cohort

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Abstract

Aims: To determine whether obesity-associated metabolites are associated with type 2 diabetes (T2DM) risk among South Asians.

Materials and Methods: Serum-based nuclear magnetic resonance imaging metabolomics data were generated from two South Asian population-based prospective cohorts from Karachi, Pakistan: CARRS1 (N = 4017) and CARRS2 (N = 4802). Participants in both cohorts were followed up for 5 years and incident T2DM was ascertained. A nested case-control study approach was developed to select participants from CARRS1 ($N_{cases} = 197$ and $N_{controls} = 195$) and CARRS2 ($N_{cases} = 194$ and $N_{controls} = 200$), respectively. First, we investigated the association of 224 metabolites with general obesity based on body mass index and with central obesity based on waist-hip ratio, and then the top obesity-associated metabolites were studied in relation to incident T2DM.

Results: In a combined sample of the CARRS1 and CARRS2 cohorts, out of 224 metabolites, 12 were associated with general obesity and, of these, one was associated with incident T2DM. Fifteen out of 224 metabolites were associated with central obesity and, of these, 10 were associated with incident T2DM. The higher level of total cholesterol in high-density lipoprotein (HDL) was associated with reduced T2DM risk (odds ratio [OR] 0.68, 95% confidence interval [CI] 0.53, 0.86; $P = 1.2 \times 10^{-3}$), while higher cholesterol esters in large very-low-density lipoprotein (VLDL) particles were associated with increased T2DM risk (OR 1.90, 95% CI 1.40, 2.58; $P = 3.5 \times 10^{-5}$).

Conclusion: Total cholesterol in HDL and cholesterol esters in large VLDL particles may be an important biomarker in the identification of early development of obesity-associated T2DM risk among South Asian adults.

KEYWORDS

observational study, population study, type 2 diabetes, body composition, dyslipidaemia, cohort study

1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) and its related sequelae are major causes of morbidity and mortality in South Asian countries, with Pakistan being the seventh highest-ranking country in terms of the global burden of diabetes.[1] It has been estimated that almost 20% of individuals aged 40 years and older have T2DM in Pakistan.[1,2] The reasons behind this increased rapid rise and large burden of T2DM in Pakistan are not well understood. In addition, T2DM onset,[3,4] risk factors for metabolic diseases[5] and related disease complications[6] occur at a younger age and at lower body mass indices (BMIs) in South Asian people, including Pakistanis, compared to other ethnic groups.[6–8] Moreover, compared with White people, South Asian people rapidly progress from prediabetes to T2DM.[9]

Type 2 diabetes mellitus is generally characterized by a gradual progression period before the onset of disease phenotype.[10,11] Substantial evidence shows that higher BMI, a measure of general obesity, and waist-hip ratio (WHR), a measure of central obesity, are the major risk factors for many chronic diseases including T2DM.[12,13] The biological mechanisms linking obesity with T2DM are not very well understood.[12,14] Comprehensive knowledge about the metabolites associated with intermediate and end-stage disease may elucidate the pathogenesis of T2DM and could help in prevention efforts.

Metabolomics could potentially facilitate the discovery of obesity-associated metabolic factors that could be important for predicting T2DM risk. Metabolomic profiling has been used to identify the metabolic signatures associated with higher obesity,[15–17] and these metabolomic biomarkers were later used to predict future risks of diabetes.[18–21] However, most of these studies have been conducted among European populations and the relevance of these metabolites has not been studied in other populations including South Asian cohorts. The metabolomic signatures may be different in this population and differences may also exist in ethnic-specific T2DM risk.

We therefore investigated the associations between circulating metabolites in relation to general as well as central obesity and whether these obesity associated metabolites are predictive of future risk of T2DM in a population-based prospective cohort of South Asians.

2 | METHODS

2.1 | Study population

The Centre for Cardiometabolic Risk Reduction in South Asia (CARRS) Study is a population-based prospective cohort with more than 5 years of follow-up that used multi-stage cluster random sampling to enroll 4017 adults aged 20 years and older from Karachi (Pakistan) in 2010/2011. This cohort is hereafter referred to as the CARRS1 cohort. A subsequent wave of the study, the CARRS Karachi, recruited a further 4802 participants in 2015 (using identical sampling and other methods to those used in CARRS1), and this cohort is hereafter referred to as the CARRS2 cohort. Details of the cohort sample and measurements have been described elsewhere.[22] The study population for both cohorts was representative of the city. Two adults, one man and one nonpregnant woman, aged 20 years or older, were selected from each household at baseline. All the study participants provided written consent before inclusion in the study and the research was approved by the ethics committees of Emory University, Atlanta, Georgia and Agha Khan University, Karachi, Pakistan. At baseline, 15-mL blood samples (fasting state) from each study participant were collected. Blood samples were transferred from the field site in cold chain to the central laboratory for further analysis. Sample aliquots were stored in cryo-vials at $-80\,^{\circ}C$ for further analysis. Detailed information about the cohorts and the study participants has been published separately.[22]

By applying a nested case-control epidemiological approach, from the population without diabetes at baseline, we selected 200 adults who developed incident T2DM during a 5-year follow-up period (cases) and 200 age- and sex-matched individuals who remained diabetes-free during follow-up (controls) from the CARRS1 cohort. Similarly, in the CARRS2 cohort, 200 adult participants were selected who developed T2DM during follow-up (incident cases) and 200 age- and gender matched controls who did not developed T2DM during follow-up. We measured metabolites in stored samples of all 800 participants. Fourteen participants' metabolomic measures from both CARRS 1 and CARRS 2 could not be completed and were therefore excluded; thus, the analytical sample was N = 786 (N_{cases} = 394 and $N_{controls}$ = 392).

2.2 | Obesity and cardiometabolic markers

All participants from both cohorts had weight, height, and waist and hip circumferences measured. Using these values, we calculated BMI (weight in kg over height in m^2). WHR was calculated as the ratio of waist (cm) to hip (cm). A binary measure of general obesity was created based on BMI (BMI < 25 kg/m^2 vs. BMI ≥25 kg/m^2), and central obesity was also calculated as a binary measure according to WHR (WHR <0.85 vs. WHR ≥0.85 for women and WHR ≤0.92 vs. WHR >0.92 for men). Bone mass and visceral fat composition were assessed using a digital scale (TANITA DC-240). Standard

procedures were employed to assess hip and waist circumference. Fasting plasma glucose and glycated haemoglobin (HbA1c) were measured using the glucose oxidase method.

2.3 | Lifestyle factors

For household income information, participants were asked "What is your total household income per month?", with answers of <3000, 3000-10 000, 10 001-20 000, 20 001-30 000, 30 001-40 000, 40 001-50 000, >50 000 (Pakistani rupees), "refused" or "do not know". For smoking exposure, participants were asked "Have you EVER used tobacco in any form (smoking, chewing, snuff)?" (yes or no). For alcohol consumption, participants were asked "Have you EVER used alcohol?" (yes or no). For physical activity, participants were asked "Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate [such as carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously?" (yes or no).

2.4 | T2DM case and control definition

For both the CARRS1 and CARRS2 cohorts incident diabetes cases were defined as study participants who were diabetes-free at baseline and at later follow-ups, who were noted to have plasma glucose levels ≥7.0 mmol/L and HbA1c levels ≥48 mmol/mol, or who self-reported physician-diagnosed diabetes or reported use of diabetes medications at follow-up examinations. Similarly, controls were defined as study participants that were age- and sex-matched to cases, but had fasting plasma glucose levels <5.6 mmol/L, HbA1c levels <39.0 mmol/mol, no self-reported physician-diagnosed diabetes, and no reported diabetes medication use at both baseline and at follow-up.

2.5 | Nuclear magnetic resonance-targeted metabolomics

Circulating metabolites from fasting serum were quantified using Nightingale high-throughput nuclear magnetic resonance (NMR) spectroscopy.[23] The serum samples were stored at −80°C and were thawed in a refrigerator before sample preparation. A proton NMR spectrum was obtained as the spectral signals from macromolecules and lipoprotein lipids were suppressed to increase detection of circulating amino acids. A total of 224 metabolites were quantified in mmol/L concentration using the NMR method. This metabolite panel captures a range of metabolites including amino acids, glycolysis-related metabolites, total fatty acids, total fatty acids and saturation measures, apolipoprotein subclasses, apolipoprotein particle size, apolipoproteins, cholesterol, inflammation and fluid balance. Details of the NMR method have been described previously.[20] All the metabolites passed strict quality control, the majority were detected in a larger sample (more than 90%) and we excluded samples with more than 10% missing values. The missing data were not imputed. In both

the CARRS 1 and CARRS 2 cohorts, amino acid measures were generally very high and lipoprotein levels were low, which is a typical sign of suboptimal sample storage conditions, including storage at −20°C for longer periods, which leads to protein breakage. The results were therefore interpreted cautiously. Unexpected amino acid signals for citrate, pyruvate, isopropyl alcohol, glutamine and glycerol were detected, which prevented the quantification of these metabolites. The mean success rate for metabolite detection in CARRS 2 was 93.3% and in CARRS1 it was 82.1%. Missing values for metabolites were imputed using the minimum detected values specific to each metabolite (reported previously).[24,25]

2.6 | Statistical analysis

All statistical analyses were performed using STATA 17.0 software. Participant characteristics are described in Table 1. To improve statistical power, individual data from CARRS1 and CARRS2 cohorts were combined, therefore, all the analyses were additionally adjusted for study cohort. Owing to the skewness of metabolite distributions, all metabolite concentrations were log-transformed and scaled to SD-transformation before conducting the analysis, thus, the regression coefficients were reported as per 1-SD difference in metabolite concentrations. First, we investigated the association of 224 metabolites with general obesity (BMI < 25 kg/m^2 vs. BMI ≥25 kg/m^2), then the top (ie, metabolites that had P values ≤2.22 × 10^{-4} [0.05/224] after correction for multiple testing) general obesity-associated metabolites were studied in association with incident T2DM using logistic regression analysis. Similarly, we investigated the association of 224 metabolites with central obesity (WHR <0.85 vs. WHR ≥0.85 for women and WHR ≤0.92 vs. WHR >0.92 for men), then the top metabolites (metabolites that had P values ≤2.22 × 10^{-4} [0.05/224] after correction for multiple testing) were studied in association with incident T2DM. We also performed analyses of the top general obesity- and abdominal obesity-associated metabolites in association with T2DM risk through additionally adjusting for ever-smoking, ever-alcohol intake, household income and physical activity.

Bone mass and visceral fat measures were considered as continuous variables in the regression analysis. Fasting plasma glucose and HbA1c measures were available for all study participants at baseline and were used as continuous measures.

Logistic regression analysis models were fitted to analyse the associations of metabolites as exposures and obesity and incident T2DM as outcomes. Analyses were adjusted for age and sex. With regard to the physiological influence of adiposity on the risk of T2DM, obesity (both general and central obesity) -associated metabolites were studied in correlation with fasting glucose, HbA1c, bone mass and visceral fat. We also performed sensitivity analysis using the general obesity specific cut-off for South Asian populations (BMI <23.9 vs ≥23.9 kg/m^2)[26] to analyse the association of metabolites with general obesity. We then analysed the association of the top South Asian general obesity-associated metabolites with incident T2DM. We also performed analyses for the top general as well as abdominal obesity-associated metabolites in association with T2DM risk through

TABLE 1   Baseline characteristics of the CARRS1 and CARRS2 study participants

	Karachi CARRS1				P_{trend}	Karachi CARRS2				P_{trend}
	Control (N=195)		Cases (N=197)			Control (N=200)		Cases (N=194)		
Sex: men, %	46		46			39		38		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Age, years	44.2	10.6	44.2	10.9	0.97	46.3	11.8	46.8	11.7	0.65
BMI, kg/m^2	26.0	5.3	28.7	5.7	<0.0001	25.6	6.2	28.0	6.3	0.0003
WHR	0.9	0.1	0.9	0.1	0.0001	0.9	0.1	0.9	0.1	0.0009
Bone mass, kg	2.4	0.6	2.5	0.5	0.16	2.6	2.6	2.6	1.8	0.94
Visceral fat, kg	7.3	3.8	8.7	4.2	0.002	8.1	4.9	9.6	5.1	0.004
Fasting glucose, mmol/L	5.0	0.46	5.3	0.58	<0.0001	4.7	0.4	5.1	0.65	<0.0001
HbA1c, mmol/mol	36	1.2	40	1.2	<0.0001	36	1.2	40	1.2	<0.0001

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; WHR, waist-hip ratio.

additionally adjusting for ever-smoking, ever-alcohol intake, household income and physical activity.

3 | RESULTS

The baseline characteristics of the Karachi CARRS1 (N = 392) and Karachi CARRS2 (N = 394) study participants (incident T2DM cases and controls) are shown in Table 1. The study population represents men and women who were of comparable age. Compared to controls, cases at baseline had significantly higher mean BMI, higher WHR, greater visceral fat, and higher fasting glucose and HbA1c levels. The general characteristics of the study participants without metabolomics data were matched to those who did have metabolomics data and were selected as a nested case-control sample (Table S8).

3.1 | Circulating metabolites associated with general obesity measures

Of the total 224 metabolites measured in the CARRS cohorts, 12 were associated with general obesity (BMI ≤25 vs. >25 kg/m^2) at $P = 2.22 \times 10^{-4}$ (0.05/224), corrected for multiple testing. Three metabolites were associated with increased general obesity, while nine were associated with reduced general obesity (Table S1). Tyrosine was strongly associated with higher risk of general obesity (odds ratio [OR] 2.87, 95% confidence interval [CI] 1.97 to 4.17; $P = 3.4 \times 10^{-8}$), while mean diameter of HDL particle was strongly associated with reduced risk of general obesity (OR 0.60, 95% CI 0.48 to 0.76; $P = 1.9 \times 10^{-5}$).

3.2 | General obesity-associated metabolites in relation to T2DM

The associations between 12 general obesity-associated metabolites and incident T2DM was investigated using logistic regression models. In the combined sample of the CARRS1 and CARRS2 Karachi cohorts,

one out of 12 general obesity-associated metabolites was significantly associated (at $P = 4.2 \times 10^{-3}$, corrected for multiple testing through 0.05/12) with incident T2DM (Table 2). Total cholesterol in HDL was associated with reduced T2DM risk (OR 0.68, 95% CI 0.53, 0.86; $P = 1.2 \times 10^{-3}$ [Table 2 and Figure 1]). We also performed sensitivity analysis of general obesity-associated top metabolites in association with T2DM while additionally adjusting for BMI (Table S3) and observed directionally similar results, although the magnitude of effects was attenuated, which shows that the metabolite-T2DM associations are not potentially confounded by BMI. We observed that additional adjustment for ever-smoking, ever-alcohol intake, household income and physical activity did not attenuate the results for general obesity (Table 2) and abdominal obesity (Table 3) -associated metabolites in relation to T2DM risk.

3.3 | Circulating metabolites associated with central obesity measures

Of the 224 metabolites, 15 were associated with central obesity (WHR <0.85 vs. WHR ≥0.85 for women and WHR ≤0.92 vs. WHR >0.92 for men [Table S2] at $P = 2.22 \times 10^{-4}$ [0.05/224]) corrected for multiple testing. All metabolites that were associated with increased central obesity are reported in Table S2. Free cholesterol in chylomicrons and presence of extremely large very-low-density lipoprotein (VLDL) particle was strongly associated with higher risk of central obesity (OR 1.65, 95% CI 1.31 to 2.07; $P = 1.5 \times 10^{-5}$).

3.4 | Central obesity-associated metabolites in relation to type 2 diabetes
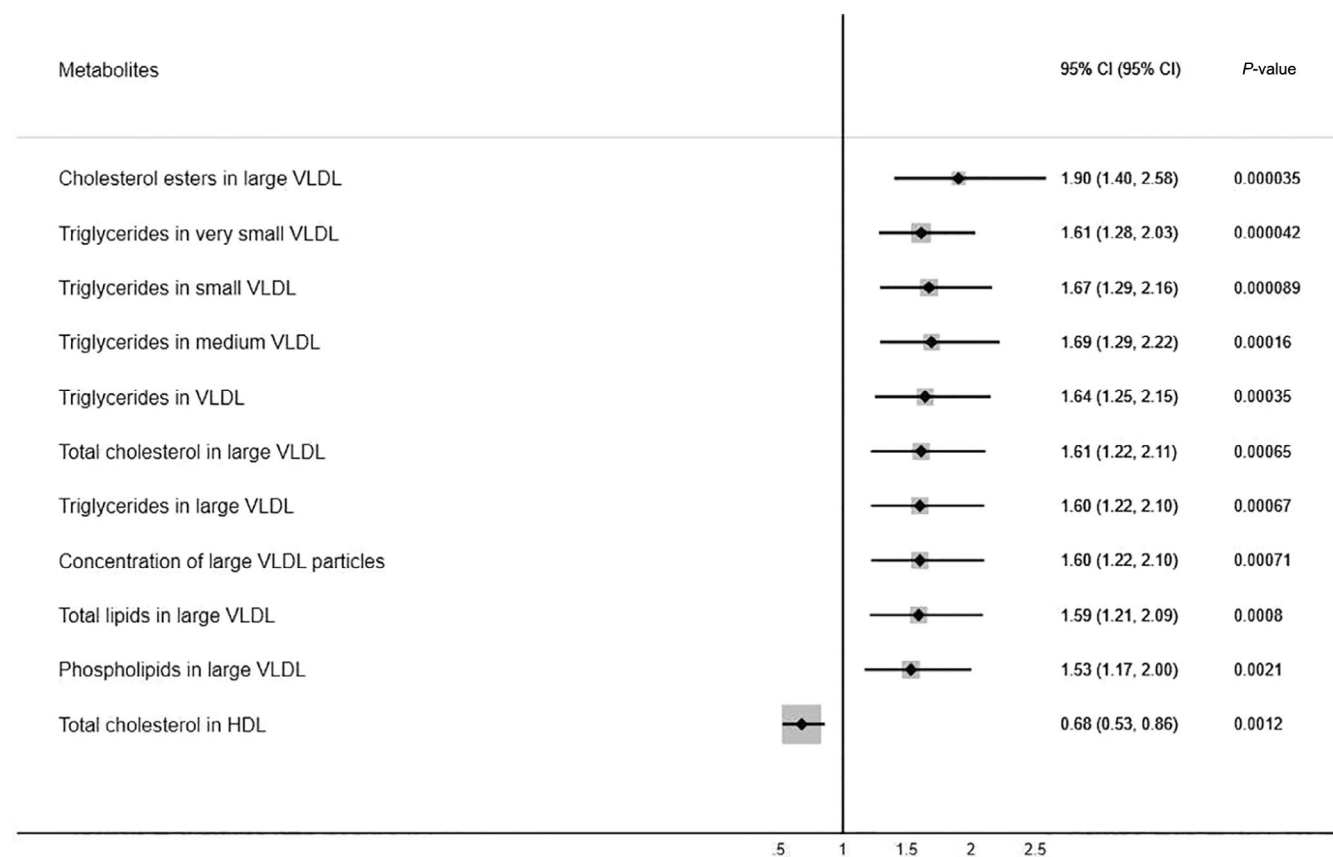
The associations between 15 central obesity-associated metabolites and incident T2DM was investigated using logistic regression models. In the combined sample of the CARRS1 and CARRS2 Karachi cohorts, 10 out of 15 central obesity-associated metabolites were significantly

TABLE 2 General obesity-associated metabolites in relation to type 2 diabetes among the combined sample of CARRS1 and CARRS2 cohorts

Biomarker	Basic model		Fully adjusted model	
	OR (95% CI)	P value	OR (95% CI)	P value
Total cholesterol in HDL	0.68 (0.53, 0.86)	1.2×10^{-3}	0.68 (0.54, 0.86)	1.6×10^{-3}
Phospholipids in very large HDL	0.78 (0.63, 0.98)	3.1×10^{-2}	0.79 (0.63, 0.99)	3.7×10^{-2}
Concentration of very large HDL particles	0.79 (0.64, 0.99)	3.8×10^{-2}	0.80 (0.64, 0.99)	4.5×10^{-2}
Total lipids in very large HDL	0.79 (0.64, 0.99)	3.8×10^{-2}	0.80 (0.64, 0.99)	4.5×10^{-2}
Tyrosine	1.35 (0.99, 1.84)	5.9×10^{-2}	1.36 (1.00, 1.87)	5.2×10^{-2}
Free cholesterol in very large HDL	0.81 (0.65, 1.02)	6.9×10^{-2}	0.82 (0.66, 1.02)	7.7×10^{-2}
Total cholesterol in very large HDL	0.82 (0.66, 1.02)	7.3×10^{-2}	0.82 (0.66, 1.02)	8.1×10^{-2}
Mean diameter for HDL particles	0.86 (0.70, 1.06)	1.5×10^{-1}	0.87 (0.70, 1.07)	1.8×10^{-1}
Total cholesterol to total lipids ratio in small HDL	0.88 (0.72, 1.09)	2.4×10^{-1}	0.87 (0.69, 1.09)	2.3×10^{-1}
Valine	0.87 (0.69, 1.10)	2.4×10^{-1}	0.88 (0.72, 1.09)	2.4×10^{-1}
Isoleucine	0.95 (0.76, 1.18)	6.4×10^{-1}	0.95 (0.76, 1.18)	6.3×10^{-1}
Total cholesterol to total lipids ratio in medium HDL	0.95 (0.77, 1.18)	6.7×10^{-1}	0.96 (0.78, 1.19)	7.2×10^{-1}

Basic models were adjusted for age and gender. Fully adjusted models were adjusted for basic model + smoking, physical activity, alcohol and income levels.

Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; OR, odds ratio.

Metabolites		95% CI (95% CI)	P-value
Cholesterol esters in large VLDL		1.90 (1.40, 2.58)	0.000035
Triglycerides in very small VLDL		1.61 (1.28, 2.03)	0.000042
Triglycerides in small VLDL		1.67 (1.29, 2.16)	0.000089
Triglycerides in medium VLDL		1.69 (1.29, 2.22)	0.00016
Triglycerides in VLDL		1.64 (1.25, 2.15)	0.00035
Total cholesterol in large VLDL		1.61 (1.22, 2.11)	0.00065
Triglycerides in large VLDL		1.60 (1.22, 2.10)	0.00067
Concentration of large VLDL particles		1.60 (1.22, 2.10)	0.00071
Total lipids in large VLDL		1.59 (1.21, 2.09)	0.0008
Phospholipids in large VLDL		1.53 (1.17, 2.00)	0.0021
Total cholesterol in HDL		0.68 (0.53, 0.86)	0.0012

FIGURE 1 Forest plot showing general obesity- and abdominal obesity-associated metabolites that were significantly associated with type 2 diabetes risk

associated (at $P = 3.3 \times 10^{-3}$, corrected for multiple testing through 0.05/15) with incident T2DM (Table 3). All 10 central obesity-associated metabolites were associated with higher risk of T2DM.

Cholesterol esters in large VLDL particles was associated with higher T2DM risk (OR 1.90, 95% CI 1.40, 2.58; $P = 3.5 \times 10^{-5}$ [Table 3 and Figure 1]).

TABLE 3 Central obesity-specific metabolites in relation to type 2 diabetes in the combined CARRS1 and CARRS2 cohort

Biomarker	Basic model		Fully adjusted model	
	OR (95% CI)	P value	OR (95% CI)	P value
Cholesterol esters in large VLDL	1.90 (1.40, 2.58)	3.5×10^{-5}	1.89 (1.39, 2.56)	4.3×10^{-5}
Triglycerides in very small VLDL	1.61 (1.28, 2.03)	4.2×10^{-5}	1.61 (1.28, 2.02)	5.5×10^{-5}
Triglycerides in small VLDL	1.67 (1.29, 2.16)	8.9×10^{-5}	1.68 (1.30, 2.17)	8.0×10^{-5}
Triglycerides in medium VLDL	1.69 (1.29, 2.22)	1.6×10^{-4}	1.69 (1.29, 2.22)	1.7×10^{-4}
Triglycerides in VLDL	1.64 (1.25, 2.15)	3.5×10^{-4}	1.65 (1.26, 2.16)	3.1×10^{-4}
Total cholesterol in large VLDL	1.61 (1.22, 2.11)	6.5×10^{-4}	1.61 (1.23, 2.11)	6.2×10^{-4}
Triglycerides in large VLDL	1.60 (1.22, 2.10)	6.7×10^{-4}	1.61 (1.22, 2.11)	6.5×10^{-4}
Concentration of large VLDL particles	1.60 (1.22, 2.10)	7.1×10^{-4}	1.60 (1.22, 2.11)	6.8×10^{-4}
Total lipids in large VLDL	1.59 (1.21, 2.09)	8.0×10^{-4}	1.60 (1.22, 2.10)	7.6×10^{-4}
Phospholipids in large VLDL	1.53 (1.17, 2.00)	2.1×10^{-3}	1.54 (1.17, 2.02)	2.0×10^{-3}
Mean diameter for VLDL particles	1.36 (1.09, 1.70)	6.1×10^{-3}	1.37 (1.10, 1.71)	5.8×10^{-3}
Free cholesterol in chylomicrons and extremely large VLDL	1.32 (1.07, 1.63)	1.1×10^{-2}	1.32 (1.06, 1.64)	1.2×10^{-2}
Triglycerides to total lipids ratio in very large HDL	1.26 (1.02, 1.55)	2.9×10^{-2}	1.26 (1.02, 1.55)	3.0×10^{-2}
Free cholesterol to total lipids ratio in large VLDL	1.23 (1.00, 1.52)	4.7×10^{-2}	1.23 (1.00, 1.52)	5.1×10^{-2}
Triglycerides to total lipids ratio in very large VLDL	1.15 (0.93, 1.41)	2.0×10^{-1}	1.14 (0.92, 1.41)	2.3×10^{-1}

Regression models were adjusted for age, sex and cohort.
Abbreviations: CI, confidence interval; OR, odds ratio; VLDL, very-low-density lipoprotein.
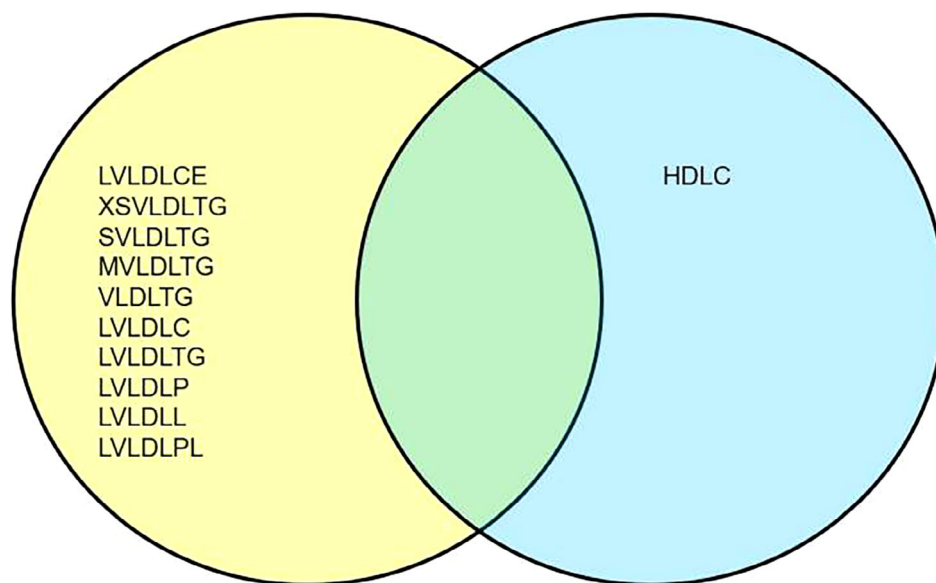
FIGURE 2 Venn diagram showing the obesity (general and abdominal) -associated metabolites in relation type 2 diabetes (T2DM). Yellow circle shows abdominal obesity-associated metabolite, blue circle shows general obesity-associated metabolites in association with type 2 diabetes risk, and green part shows shared metabolites between general and abdominal obesity. Abbreviations: HDLC, total cholesterol in HDL; LVLDLCE, cholesterol esters in large VLDL; XSVLDLTG, triglycerides in very small VLDL; SVLDLTG, triglycerides in small VLDL; MVLDLTG, triglycerides in medium VLDL; VLDLTG, triglycerides in VLDL; LVLDLC, total cholesterol in large VLDL; LVLDLTG, triglycerides in large VLDL; LVLDLP, concentration of large VLDL particles; LVLDLL, total lipids in large VLDL; LVLDLPL, phospholipids in large VLDL

We also performed sensitivity analysis by testing the association of the top central obesity-associated metabolite associations with T2DM while additionally adjusting for WHR (Table S4) and observed directionally similar results, although the significance of the association was attenuated. None of the metabolites were shared between the general as well as abdominal obesity in association with T2DM risk after correction for multiple testing (Figure 2).

3.5 | Obesity-specific metabolites in correlation with cardiometabolic factors

All obesity-associated metabolites (identified through both general as well as central obesity) that were significantly associated with risk of T2DM were investigated further by examining their correlation with traditional cardiometabolic risk factors including bone mass, visceral fat, fasting glucose and HbA1c in the combined sample (Table 4). Total cholesterol in HDL particle was negatively correlated with bone mass ($r = -0.10$, $P = 0.011$), visceral fat ($r = -0.09$, $P = 0.015$), and HbA1c ($r = -0.07$, $P = 0.04$) but was not correlated with fasting glucose ($r = -0.07$, $P = 0.07$). While most of the other discovered metabolites were positively correlated with these biomarkers (Table 4).

3.6 | Sensitivity analysis

Using a South Asian-specific general obesity cut-off, we observed that 11 metabolites were associated with general obesity ($P = 2.24 \times 10^{-04}$ [Table S5]). We then analysed the association of these South Asian obesity-specific metabolites with incident T2DM and observed that 1 out 11 at P value: 4.5×10^{-03} of the metabolites were associated with incident T2DM (Table S6). Similarly to the European population-specific

general obesity (BMI less than equal 25 kg/m^2 vs BMI >25 kg/m^2) associated T2DM metabolites, we observed that total cholesterol in HDL was associated with incident T2DM. Correlation analyses between the top general obesity- as well as abdominal obesity-associated metabolites in relation to T2DM risk showed that the metabolites were generally correlated with each others (Table S7).

4 | DISCUSSION

This first large-scale study in a South Asian population using NMR-based metabolomics showed that general obesity- as well as central obesity-associated metabolomic signatures were associated with future risk of T2DM. Out of 224 tested metabolites, 12 were associated with general obesity and, of these, one metabolite was associated with risk of T2DM. Similarly, out of 224 metabolites, 15 were associated with central obesity and, of these, 10 were associated with increased risk of T2DM. A key finding of the present study was that circulating metabolites, including total cholesterol in HDL, were associated with less general adiposity and reduced risks of T2DM. Further analysis highlighted that total cholesterol in HDL metabolite was negatively correlated with bone mass, visceral fat and HbA1c, but not with fasting glucose. However, presence of triglyceride-rich VLDL

TABLE 4 Correlations between cardiometabolic biomarkers and type 2 diabetes-specific metabolites in the combined sample of CARRS1 and CARRS2 cohorts

		Bone mass	Visceral fat	Fasting glucose	HbA1c
Total cholesterol in HDL	r	−0.10	−0.09	−0.07	−0.07
	P value	0.011	0.015	0.07	0.04
Cholesterol esters in large VLDL	r	0.01	0.18	0.16	0.21
	P value	0.77	<0.0001	<0.0001	<0.0001
Triglycerides in very-small VLDL	r	−0.004	0.15	0.10	0.14
	P value	0.91	1.0×10^{-4}	7.4×10^{-3}	1.0×10^{-4}
Triglycerides in small VLDL	r	0.01	0.16	0.14	0.16
	P value	0.79	<0.0001	1.0×10^{-4}	<0.0001
Triglycerides in medium VLDL	r	0.02	0.17	0.17	0.20
	P value	0.53	<0.0001	<0.0001	<0.0001
Triglycerides in VLDL	r	0.03	0.174	0.1651	0.19
	P value	0.50	<0.0001	<0.0001	<0.0001
Total cholesterol in large VLDL	r	0.02	0.18	0.16	0.19
	P value	0.67	<0.0001	<0.0001	<0.0001
Triglycerides in large VLDL	r	0.03	0.19	0.17	0.19
	P value	0.45	<0.0001	<0.0001	<0.0001
Concentration of large VLDL particles	r	0.02	0.19	0.16	0.19
	P value	0.51	<0.0001	<0.0001	<0.0001
Total lipids in large VLDL	r	0.02	0.18	0.16	0.19
	P value	0.53	<0.0001	<0.0001	<0.0001
Phospholipids in large VLD	r	0.02	0.18	0.16	0.19
	P value	0.57	<0.0001	<0.0001	<0.0001

Abbreviations: HbA1c, glycated haemoglobin; VLDL, very-low-density lipoprotein.

small, medium, large and very-large particles were associated with increased central obesity as well as higher risk of T2DM. Further analysis showed that these metabolites were positively correlated with bone mass, visceral fat, fasting glucose and HbA1c.

Metabolomics has been used to explore changes in metabolism that are associated with future onset of diabetes.[18] These changes that are present many years before the clinical manifestations might help in assessing an individual's risk of developing T2DM but are not necessarily related to health outcomes in individuals with overt diabetes. We observed that increased levels of circulating triglyceride-rich small and medium VLDL particles were associated with increased general obesity and higher risk of T2DM among South Asians. Similar findings have been observed among Finnish populations.[20] Higher level of triglycerides in small and medium HDL particles reflect early aberrations in lipoprotein metabolism, which is a characteristic of insulin resistance including increased catabolism of HDL particles and increased transfer of triglycerols to HDL particles. Consistent with the Finnish study findings,[20] we have shown that presence of triglyceride-rich VLDL small particles were positively correlated with bone mass, visceral fat, central obesity, fasting glucose and HbA1c. Girona et al have also reported that triglyceride-rich VLDL particles were associated with metabolic syndrome.[27]

Abdominal obesity-associated metabolites that were significantly associated with T2DM risk were enriched for small, large and very-large VLDL particles, and VLDL particle pathways. Our findings that total cholesterol in HDL was associated with reduced risk of T2DM are consistent with the previous literature.[28-30] Previous animal and human studies suggest that increasing plasma HDL cholesterol levels may modulate insulin secretion.[31] Our results might suggest that future interventions of increasing circulating HDL levels of HDL levels might be a promising approach to preventing diabetes.

This is the first population-based study of South Asians living in Pakistan to investigate the role of metabolites in association with future risk of T2DM with a prospective nested design and longitudinal follow-up. The study was conducted in a representative sample of middle-aged men and women in a large urban setting in Pakistan, so the findings are more generalizable than those obtained from a clinical setting alone. The study also has some limitations, including the fact that we did not have baseline measures of fasting insulin, therefore, we could not explore insulin sensitivity and insulin resistance or how circulating metabolite levels are associated with those physiological markers. The associations observed in this study between metabolites and incident diabetes were based on small numbers and should be interpreted with caution. We measured metabolite profiles only at baseline. This might lead to exposure misclassification in a random manner, which might lead to the attenuation of associations between metabolites and diabetes. We also observed that the ratio of triglycerides to total lipids in small HDL particles, and triglycerides in very-small, small, medium and large VLDL particles were associated with both increased general and central obesity and increased risk of T2DM. These metabolites highlight novel potential pathophysiological links between central obesity and diabetes. These data also indicate that fasting glucose and T2DM-related metabolites may be different from HbA1c-related metabolites.

In summary, this prospective nested case-control study in South Asian adults demonstrates that serum concentration obesity-associated total cholesterol in HDL metabolites were associated with decreased risks of incident T2DM. Meanwhile, obesity-associated triglyceride-rich small, medium, large and very-large VLDL particles were associated with increased risks of T2DM. These obesity-associated metabolites may be important early biomarkers for the identification of people at higher risk of developing T2DM before overt symptoms and for understanding the previously unknown pathways in T2DM pathophysiology. Our study provides important signals that obesity-associated metabolites may be associated with future T2DM risk. These associations need to be replicated in larger samples and in subgroups of South Asians with different socioeconomic[32] and sociodemographic characteristics (eg, in different intra-South Asian ethnicities) to better understand T2DM pathophysiology.

CONFLICT OF INTEREST

None of the authors declare any potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14788.

DATA AVAILABILITY STATEMENT

The raw data are not publicly available owing to the study's data access policies. However, analyses in the CARRS dataset can be requested by contacting the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.