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Lipoproteins and lipoprotein lipid composition are associated with stages of dysglycemia and subclinical coronary atherosclerosis

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ABSTRACT

Background: Dyslipidaemia in patients with diabetes contributes to the risk of atherosclerotic cardiovascular disease. We aimed to identify a dyslipidemic profile associated with both dysglycemia and subclinical coronary atherosclerosis.

Methods: Study participants (n = 5050) were classified in three groups: normoglycemia, pre-diabetes, and diabetes. A coronary artery calcium score (CACS) > 0 defined subclinical coronary atherosclerosis. Two independent methods were used to identify, among 225 lipid biomarkers, those that were associated with pre-diabetes and diabetes and were further tested for association by zero inflated Poisson regression with CACS and with CACS burden in study participants with CACS>0. Estimates were adjusted for cardiovascular risk factors with an interaction term for dispensed lipid lowering drugs.

Abbreviations: ASCVD, Atherosclerosis related cardiovascular disease; BMI, Body Mass Index; CACS, Coronary artery calcium score; CI, Confidence Interval; DBP, Diastolic Blood Pressure; FA, Fatty Acids; HDL, High Density Lipoprotein; HS, High sensitivity; IDL, Intermediate Density Lipoprotein; LDL, Low Density Lipoprotein; MUFA, Mono Unsaturated Fatty Acids; NMR, Nuclear Magnetic Resonance; OR, Odds Ratio; PA, Physical activity; PUFA, Poly Unsaturated Fatty Acids; RR, Rate Ratio; SCAPIS, Swedish Cardiopulmonary Bioimage Study; SBP, Systolic Blood Pressure; SES, Socioeconomic status; SHAP, SHapley Additive exPlanations; VLDL, Very Low Density Lipoprotein.

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Results: Thirty-two biomarkers associated with prediabetes and diabetes were further investigated for association with CACS. HDL diameter [multi-adjusted OR of 0.85 and 95 %CI (0.78–0.92)] as well as free cholesterol, phospholipids and total lipids in extra large HDL were inversely associated with CACS. There was a borderline significant interaction between small HDL and dispensed lipid lowering drugs on the presence of CACS, with and multi-adjusted OR of 0.53 and 95 %CI (0.36–0.77). None of the 32 glycemic profile-related lipid biomarkers associated with the relative increase of CACS in those with CACS>0. No consistent association was observed between non-HDL lipoproteins and CACS.
Conclusions: Changes in composition and relative concentration of HDL associated with both dysglycemia and subclinical coronary atherosclerosis. Treatment with lipid lowering drugs may contribute to reduce the risk associated with high circulating levels of small HDL.

1. Introduction

Derangements of both glucose and lipid metabolism characterize the clinical course of diabetes and increase the risk for atherosclerotic cardiovascular disease (ASCVD), the most common cause of death in persons with diabetes [1,2].

Individuals with both pre-diabetes and diabetes have different degree of dyslipidaemia. Typically, high circulating levels of triglycerides [3], low HDL-cholesterol levels, high small dense LDL with normal or slightly elevated total- and LDL-cholesterol levels are observed in individuals with diabetes. However, lipids, lipoproteins circulating levels and their composition differ greatly in individuals with diabetes depending on stage of the disease, metabolic compensation and type of treatment [4–8]. Observational [9] and interventional studies have shown, in fact, that LDL-cholesterol levels may even be lower in individuals with diabetes as compared to individuals without diabetes [4,5].

As dyslipidaemia is a major ASCVD risk factor and a hallmark of the metabolic disease associated with diabetes, identification of lipid, lipoprotein and related metabolites able to discriminate individuals with pre-diabets and diabetes at high risk of ASCVD, is fundamental for implementation of precision medicine.

To this end, we aimed to identify a dyslipidaemic profile based on lipid and lipoproteins concentration, lipoprotein lipid content, and metabolites able to discriminate individuals across three glycaemic categories, defined as normoglycemia, prediabetes, and diabetes in middle aged men and women without overt ASCVD. Further, we investigated if the glycemic profile-related lipid biomarkers were associated with the presence of coronary artery calcium (CAC), a measure of subclinical atherosclerosis. In this latter analysis, we estimated the effect of the interaction between biomarkers and dispensed lipid lowering drugs, as hypolipidemic drugs are a mainstay in ASCVD prevention in individuals with diabetes.

2. Methods

2.1. Study population

The Swedish Cardiopulmonary Bioimage Study (SCAPIS) is described in detail elsewhere [10,11]. Briefly, 30,000 study participants (50–64 years old, 50 % women) were recruited through a random selection from the Swedish population register at six Swedish university hospitals (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå and Uppsala), between 2014 and 2018. All study participants filled in a comprehensive health questionnaire and underwent a physical examination. Blood samples were collected and stored at −80 °C until analysis. In this study, we included a subgroup of the SCAPIS consisting of 5050 randomly selected study participants with extensive molecular phenotyping (SCAPIS-OMICS).

For the present analysis, we excluded study participants with self-reported ASCVD: ischemic heart disease (n = 127), ischemic stroke in the absence of known atrial fibrillation (n = 66) and peripheral artery disease (PAD; n = 16). Further, we excluded study participants with left or right ankle-brachial index (ABI) < 0.9 at the physical examination (n = 67), as well as those with missing values for >50 % of the metabolites and/or biochemical values (n = 6). After exclusions, 4773 study participants were included in the analysis. Supplementary Fig. I summarizes the inclusion and exclusion criteria. Details on the definition of the demographic, anthropometric, and cardiovascular risk factors are reported in the Supplementary Material.

Circulating levels of glucose, HbA1c, lipids and high sensitive (hs) C-reactive protein were measured in plasma after overnight fasting using standard methods at the laboratory at each participating university hospital. Information about dispensed lipid-lowering drug was obtained from the Swedish Prescribed Drug Register using the ATC code C10.

2.2. Ethics

SCAPIS was approved by the ethical review board at Umeå University, Sweden (Dnr 2010–228-31 M and Dnr 2017–183-31 M). All study participants provided an informed written consent before enrolment in the study.

2.3. Outcomes

Glycemic status was defined as normoglycemia if fasting plasma glucose was <6.1 mmol/L and HbA1c <6.0 % (<42 mmol/mol); as prediabetes if fasting plasma glucose was 6.1–6.9 mmol/L or HbA1c 6.0–6.5 % (42–47 mmol/mol); as diabetes if fasting plasma glucose was≥7.0 mmol/L or HbA1c >6.5 % (≥48 mmol/mol) and/or self-reported diabetes [11,12].

Subclinical coronary atherosclerosis was estimated by CAC on computer tomography scans following a pre-specified protocol [10,13] and scored using the syngo.via calcium scoring software (Siemens-Erlangen- Germany) [13]. The area of calcification of each 3 mm slice was multiplied by an intensity factor and summed up to a CAC score (CACS) for the artery tree according to Agatston [14]. Presence of CAC was defined if CACS>0.

2.4. Measurement of circulating levels of lipids, lipoproteins, lipoprotein lipid content, lipid metabolites and amino acids

Plasma levels of lipid lipoproteins, lipoprotein lipid content, lipid metabolites and aminoacids were measured by nuclear magnetic resonance (NMR) spectroscopy [15] on a panel provided by Nightingale Health (Helsinki- Finland; nightingalehealth.com).

The Nightingale panel used in the present study consists of (1) absolute measurements of lipids, lipoproteins, lipoprotein lipid components (total and, free cholesterol, cholesterol esters, phospholipids, triglycerides and total lipids), small metabolites and aminoacids, all reported in mmol/L and (2) relative measures of the lipid content of each circulating lipoprotein expressed as ratio of lipoprotein lipid content vs the total pool of lipids in the lipoprotein. Altogether 225 measurements (hereby lipid biomarkers) were available. Supplementary Table I summarizes the classes, numbers, and sizes of the lipids, lipoprotein and aminoacids, measured on this panel.

Circulating plasma levels (mmol/L) of triglycerides, total, LDL and HDL cholesterol were also measured in plasma after overnight fasting using standard methods at the laboratory at each participating university hospital.

2.5. Statistical analysis

Continuous variables are reported as median and interquartile range (IQR) and categorical as number (percentage).

We used two independent strategies to identify lipid biomarkers associated with the three glycemic profiles as summarized in Supplementary Fig. II. Before analyses, metabolites were *Z*-standardized. We performed a multinomial logistic regression analysis (defined as one-by-one strategy in Supplementary Fig. II) to test the association of each one of the 224 metabolites (after the exclusion of glucose) with pre-diabetes and diabetes. The normoglycemia group was used as reference. Results are expressed as odds ratios (OR) and 95 % confidence intervals (CI) and adjusted for age, sex, socioeconomic status (SES), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), smoking, physical activity (PA), and alcohol consumption.

Up to 14 % of the study population had one or more missing values in one of the clinical or laboratory measurements. The missing values were imputed using 20 imputed datasets [16].

In parallel, we used a supervised machine learning approach by random forest and SHapley Additive exPlanations (SHAP) model (Random Forest strategy in Supplementary Fig. II). The SHAP compares the difference in model performance in a dataset with and without a specific variable, thereby measuring the contribution of each variable in the model [17]. We built a random forest model including the lipid biomarkers listed Supplementary Table I together with the set of confounders used in the multinomial logistic regression (age, sex, SES, BMI, SBP, DBP, smoking, PA, and alcohol consumption). The global SHAP ranks the variables according to the relevance of their contribution to define the outcome, the larger the SHAP value the more important is the variable). In this study, we used random forest to select the top 50 variables, as the inclusion of additional variables did not improve prediction. A Venn diagram was used to select the lipid biomarkers identified by both approaches as associated with the glycemic profiles. After adjustment for multiple comparisons using the method described by Gao et al. [18], we estimated 41 independent tests and a *p value* threshold of 0.00061 was used [18,19].

Pairwise Pearson's correlation coefficients were estimated between the lipid biomarkers associated with glycemic profiles. Additionally, we estimated the correlation coefficient for glucose and lipids measured by conventional biochemical methods and by NMR.

Zero-inflated Poisson regression was used to estimate the association between the glycemic profile-related lipid biomarkers and CACS. We performed the analysis in the complete case analysis and after multiple imputation analysis (n = 20) [16]. Zero-inflated Poisson regression models two processes simultaneously. The first one estimates the association of a glycemic profile related lipid biomarker with the presence of CACS, defined as CACS>0. Estimates are expressed as odds ratios (OR) and 95 %CI. The second model estimates the association between a one-unit increase in each one of the glycemic profile related lipid biomarkers and the relative increase in CACS value and is expressed as rate ratio (RR) and 95 %CI. We adopted three models: model 1, as univariable analysis; model 2: adjusted by age, sex, BMI, smoking, alcohol consumption, PA, SES, SBP, DBP, and model 3: model 2+ dispensed lipid lowering drugs. Additionally, analyses were performed in the entire population and after stratification by dispensed lipid-lowering drugs.

To estimate the interaction between each glycemic profile related lipid biomarker and dispensed lipid lowering drugs, we performed two interaction analyses. In the first one, we added dispensed lipid lowering drugs to model 2. In the second interaction model, we considered only dispensed statin. To this end, we classified the dispensed statin as low and high dose [20]. Low dose was defined as dose of statin reported to lower LDL-cholesterol levels by ≤30 %, i.e. fluvastatin (20–40 mg/day), pravastatin (10–40 mg/day) and simvastatin (10 mg/day). High dose statin (mg/day) was defined if the expected reduction in LDL-cholesterol was >30 %, i.e.fluvastatin (80 mg/day), simvastatin (20–40 mg/day), atorvastatin (10–80 mg/day) and rosuvastatin (5–40 mg/day).

Bootstrapping resampling was performed to estimate the robust standard error and to minimize the false positive rate. A *p value* threshold of 0.00132 was used after correction for multiple comparisons [18,19] as 19 independent tests were estimated [18].

Analyses were performed using R4.1 package.

3. Results

Study participants with diabetes (n = 261) were more likely to be men, had a higher prevalence of self-reported hyperlipidemia and hypertension as compared to those with pre-diabetes (n = 642) and normoglycemia (n = 3870). Fifteen study participants had a self-reported age of onset of diabetes before the age of 40 (2–40) and did not report use of hypoglycemic drugs. Lipid lowering drugs were dispensed to 627 (13 %) study participants (Table 1), out of which 607 received statins.

Table 1
Demographic characteristics, lifestyle habits and prevalence of cardiovascular risk factors according to the glycemic status: normoglycemia, pre-diabetes and diabetes mellitus.

	Normoglycemia (N = 3870)	Pre-diabetes (N = 642)	Diabetes mellitus (*N* = 261)
Age (years)	57 (54–61)	58 (55–62)	59 (55–62)
Female/Male, n (%)	2044 (53)/1826 (47)	298 (46)/344 (54)	99 (38)/162 (62)
Born in Sweden n (%)	3329 (86)	562 (87)	203 (78)
Education attainment, n (%)			
Up to Primary education	338 (9)	75 (11)	35 (13)
Secondary education and above	3503 (91)	567 (89)	222 (87)
Difficulties in managing regular expenses: n (%)	186 (5)	21 (3)	10 (0.4)
PA (min/wear time)			
Low intensity	2290 (1841–2754)	2287 (1782–2800)	2040 (1591–2602)
Moderate intensity	306 (212–422)	292 (208–416)	249 (156–389)
Moderate/vigorous intensity	336 (226–467)	320 (222–446)	256 (160–420)
Vigorous-intensity PA	6 (1–38)	3 (0–24)	1 (0–11)
Alcohol consumption Frequency having an alcoholic drink (last year)			
Less than or 2–4 times/months	2364 (61)	403 (63)	192 (74)
2–3 times/weeks or more	1487 (49)	236 (47)	66 (36)
Smoking n (%)	434 (11)	109 (17)	40 (15)
Cardiovascular risk factors n (%)			
Hypertension	732 (19)	189 (29)	125 (48)
Hyperlipidemia	329 (8)	96 (15)	93 (36)
Dispensed lipid lowering drugs n (%)	387 (10)	107 (16)	133 (51)

Continuous data are reported as median (IQR)- categorical variables as n and percentage. Percentage refers to the number of individuals in each group, normoglycemia, pre-diabets, diabetes mellitus.
Abbreviations: PA: Physical Activity.
Missing data: Born in Sweden n = 10, Education: n = 17; Difficulties in managing regular expenses during the last 12 months n = 28; PA n = 175; frequency of having an alcoholic drink during the last year n = 25; smoking: n = 130; cardiovascular risk factors (self-reported) n = 66.

The two most prescribed statins were atorvastatin (42 %) and simvastatin (41 %), followed by rosuvastatin (8 %), pravastatin (5 %) and fluvastatin (4 %).

We observed a progressive increase in median values for BMI, waist-hip ratio, SBP and DBP, glucose, HbA1c, and hs-CRP across the three glycemic groups, from normoglycemia to diabetes (Table II). Total-, LDL- and HDL-cholesterol levels were lower and triglycerides higher in the prediabetes and diabetes groups as compared to normoglycemia (Table 2). CAC was more prevalent in individuals with diabetes (62 %) as compared to pre-diabetes (44 %) and normoglycemia (37 %), consistently with data from the entire SCAPIS population [11]. Among those with diabetes, 24 % had a CACS>100. As shown in Table 2, no differences were observed in CACS distribution in the coronary arteries, across the three groups, with the left main/left anterior descending coronary artery bearing the highest absolute and relative CAC burden.

3.1. Lipids, lipoproteins, lipoprotein lipid content and metabolites distribution in normoglycemia, pre-diabetes and diabetes

In the Supplementary Material, we report the median value of glucose, lipids and lipoproteins measured by conventional biochemical methods (Supplemental Table II) and the distribution of small molecules, free fatty acids, lipoprotein fractions and content measured by NMR in the Supplemental Tables III to IX in the entire population and after stratification by presence/absence of dispensed lipid lowering drugs.

Table 2
Anthropometric, clinical characteristics, biochemical data and CACS in the three glycemic status groups: normoglycemia, prediabetes and diabetes mellitus.

	Normoglycemia (N = 3870)	Pre-diabetes (N = 642)	Diabetes mellitus (N = 261)
Anthropometry			
BMI, kg/m2	25.8 (23.6–28.5)	27.8 (25.3–31)	29.5 (27–33.1)
Waist-hip ratio	0.90 (0.84–0.97)	0.94 (0.88–1.0)	0.99 (0.93–1.0)
Clinical measurements			
SBP, mmHg	124 (115–136)	131 (120–142)	134 (122–144)
DBP, mmHg	78 (71–84)	81 (74–88)	80 (76–88)
Biochemistry (mmol/L)			
Fasting glucose	5.3 (4.9–5.6)	6.2 (6.1–6.5)	7.3 (6.5–8.9)
Hba1c (mmol/mol)	35 (33–37)	39 (36–42)	48 (43–57)
Fasting lipids			
Triglycerides	1.0 (0.8–1.4)	1.2 (0.9–1.6)	1.4 (0.9–1.9)
Total cholesterol	5.6 (4.9–6.2)	5.4 (4.7–6.1)	4.7 (3.9–5.6)
LDL-cholesterol	3.5 (2.9–4.1)	3.3 (2.7–3.9)	2.7 (2.1–3.5)
HDL-cholesterol	1.6 (1.3–2.0)	1.5 (1.2–1.8)	1.3 (1.1–1.6)
hsCRP, mg/L	0.9 (0.6–1.9)	1.4 (0.6–2.8)	1.7 (0.7–3.4)
Measures of subclinical atherosclerosis (Agatston Unit)			
CACS >0	1451 (37)	281 (44)	163 (62)
CACS total	29 (7.0–100)	48 (8.0–200)	56 (14–190)
CACS per coronary artery			
LM and LAD	19 (3.0–74)	31 (3.0–110)	29 (4.5–120)
LM and LAD/total CACS	0.9 (0.5–1.0)	0.8 (0.4–1.0)	0.7 (0.31.0)
Circumflex	0 (0–3.0)	0 (0–14)	0 (0–24)
Circumflex/total CACS	0 (0–0.06)	0 (0–0.10)	0 (0–0.17)
RCA	0 (0–10)	1.0 (0–32)	2.0 (0–29)
RCA/total CACS	0 (0–0.2)	0.003 (0–0.36)	0.06 (0–0.4)

Continuous data are reported as median (IQR), categorical variables as n and percentage.
CACS median values are reported for 1895 study participants with CACS>0.
Abbreviations: BMI: Body Mass Index; CACS: Coronary Artery Calcium Score; DBP: Diastolic Blood Pressure; hsCRP: high sensitivity C-reactive protein; LAD: Left anterior descending LM: left main; RCA: Right coronary artery; SBP: Systolic Blood Pressure TG: triglycerides.
Missing data: Glucose n = 3, HbA1c n = 6, Total cholesterol n = 1, LDL *n* = 38. Triglycerides and HDL-cholesterol n = 4; hsCRP *n* = 6.

3.2. Selection of the lipid biomarkers associated with pre-diabetes and diabetes

The multinomial logistic regression identified 120 out of 224 lipid biomarkers as associated with pre-diabetes and diabetes.(Supplementary Fig. III, Panel A-J).

No difference was observed between the original and imputed dataset (data not shown). Using the selection based on SHAP, 50 variables discriminated the three glycemic categories: age, sex, BMI, SBP, DBP, and 45 lipid biomarkers included in the panel (Supplementary Fig. IV).

Thirty-two lipid biomarkers were identified by the two selection processes as associated with pre-diabetes and diabetes (Supplementary Fig. V): 5 metabolites (alanine, valine, leucine, isoleucine and lactate), the mean diameter of VLDL, the concentration of HDL, the relative circulating levels of MUFA and PUFA, the relative concentration of triglycerides in extra-small, small, medium VLDL, IDL, small and large LDL ($n = 6$), the absolute concentration of total cholesterol in IDL and the relative concentration of total cholesterol in IDL, small and large VLDL ($n = 3$), the relative amount of cholesterol esters in small, large and extra-large VLDL with chylomicrons, small and large LDL(n = 6), the absolute (in medium LDL and extra-large HDL, n = 3) and the relative amount of free cholesterol in IDL, the absolute concentration of phospholipids in small and extra-large HDL($n = 2$), triglycerides in large HDL and total lipids in extra-large HDL. These 32 measurements are defined hereby as glycemic profile-related lipid biomarkers.

3.3. Glycemic profile-related lipid biomarkers: association with diabetes

Fig. 1 shows the association of the 32 glycemic profile-related lipid biomarkers. With pre-diabetes and diabetes as well as their ranking by SHAP. Fig. 2 shows the correlation matrix across these 32 biomarkers.

3.3.1. Fatty Acids (FA)

A relative excess of monounsaturated fatty acids (MUFA) (MUFA/total FA) associated with diabetes with a multi-adjusted OR of 1.40 and 95 %CI (1.18–1.67), while a relative excess of polyunsaturated fatty acids (PUFA) (PUFA/total FA) showed an inverse association with a multi-adjusted OR of 0.66 and 95 %CI (0.56–0.78). MUFA/total FA ratio was inversely correlated to lipid content in VLDL and HDL and directly correlated to VLDL triglyceride content, whilst the PUFA/total FA ratio showed an inverse pattern (Fig. 2).

3.3.2. HDL

Increasing circulating levels of small HDL associated with diabetes with an OR of 1.37 and 95 %CI of (1.15–1.63). Among the HDL lipid components, phospholipids in small HDL associated with diabetes. On the contrary, lipid rich large (triglyceride) and extra-large (free cholesterol, phospholipids, and cholesterol esters) HDL associated with a lower risk of diabetes (Fig. 1). Lipid components in large and extra-large HDL were highly correlated (Fig. 2). Phospholipids content in small HDL was highly correlated with the small HDL concentration (Fig. 2).

3.3.3. VLDL, IDL, LDL

An increase in VLDL mean diameter associated with diabetes with an OR of 1.42 and 95 %CI (1.19–1.62). A relative high triglycerides concentration in VLDL, IDL and LDL was associated with diabetes (Fig. 1), with the highest risk estimate observed for the triglyceride content in medium VLDL with an OR of 2.47 and 95 %CI (2.07–2.95). Triglyceride content in small, extra small and medium size VLDL was inversely correlated with the cholesterol and phospholipids content in HDL

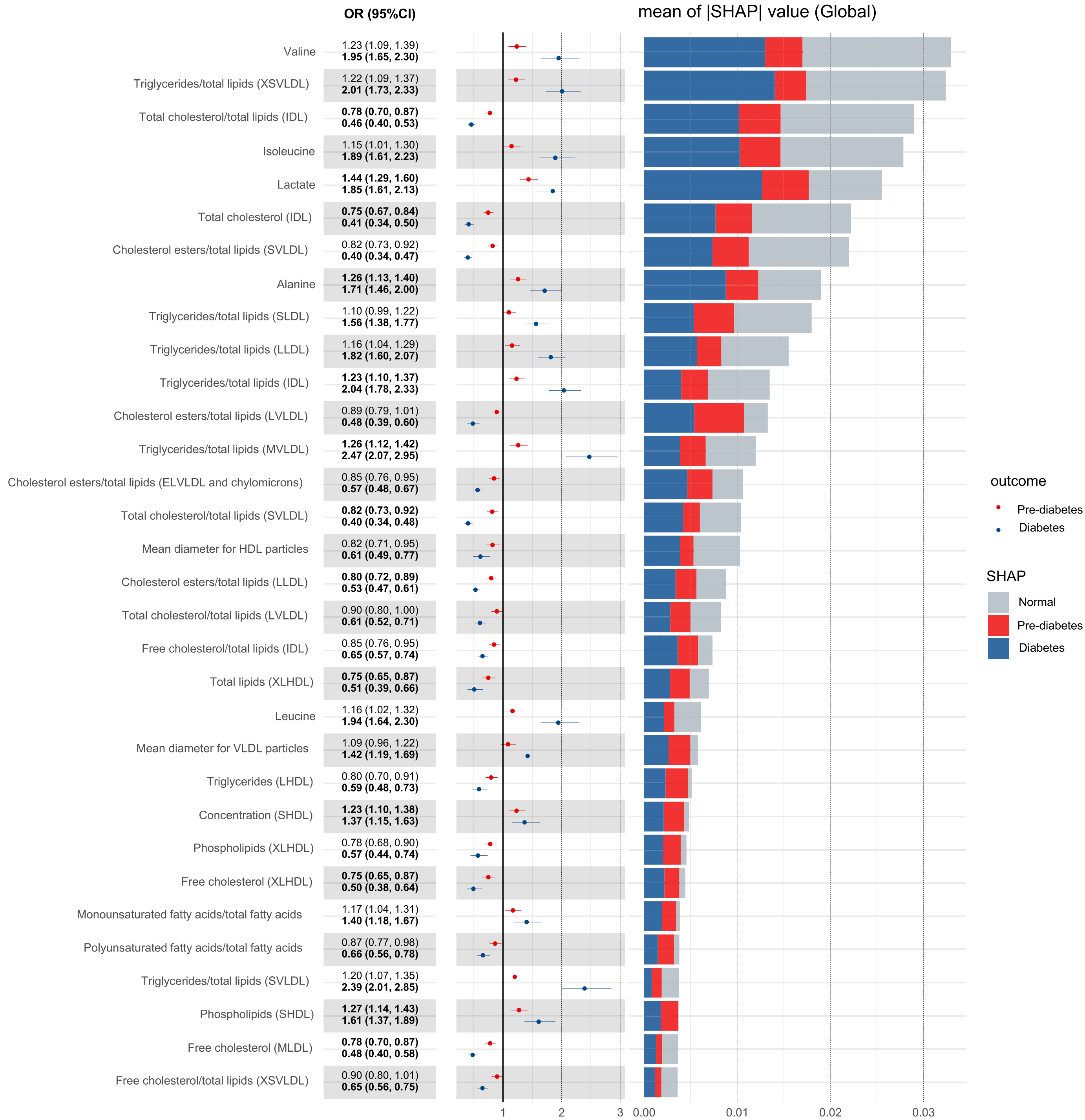

Fig. 1. Association of the 32 glycemic profile-related lipid biomarkers with pre-diabetes and diabetes.
Left panel: Representation of the results of the multinomial logistic regression showing the association of the 32 glycemic profile-related lipid biomarkers with pre-diabetes (red) and diabetes (blue) expressed as OR (95 % CI). In bold the estimates surpassing the significance threshold *p value* $\leq$ 0.00061. Right panel: results of the global SHAP. Each bar represents the value of each biomarker in predicting normoglycemia (dark grey)- pre-diabetes (red) and diabetes (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2). A relative increase in the concentration of cholesterol esters in small, large, extra-large with chylomicrons VLDL associated with a lower risk of diabetes, the lowest risk estimate being observed for cholesterol ester/total lipids ratio in small VLDL with an OR of 0.40 and 95 %CI (0.34–0.47).

3.3.4. *Small molecules*

High circulating levels of valine, alanine, isoleucine, leucine and lactate were strongly associated with diabetes (Fig. 1) and inversely correlated with the HDL lipid content (Fig. 2).

3.4. *Glycemic profile-related lipid biomarkers: association with pre-diabetes*

The presence of pre-diabetes was associated with a lipid and metabolic profile similar to the one observed in diabetes, with some of the

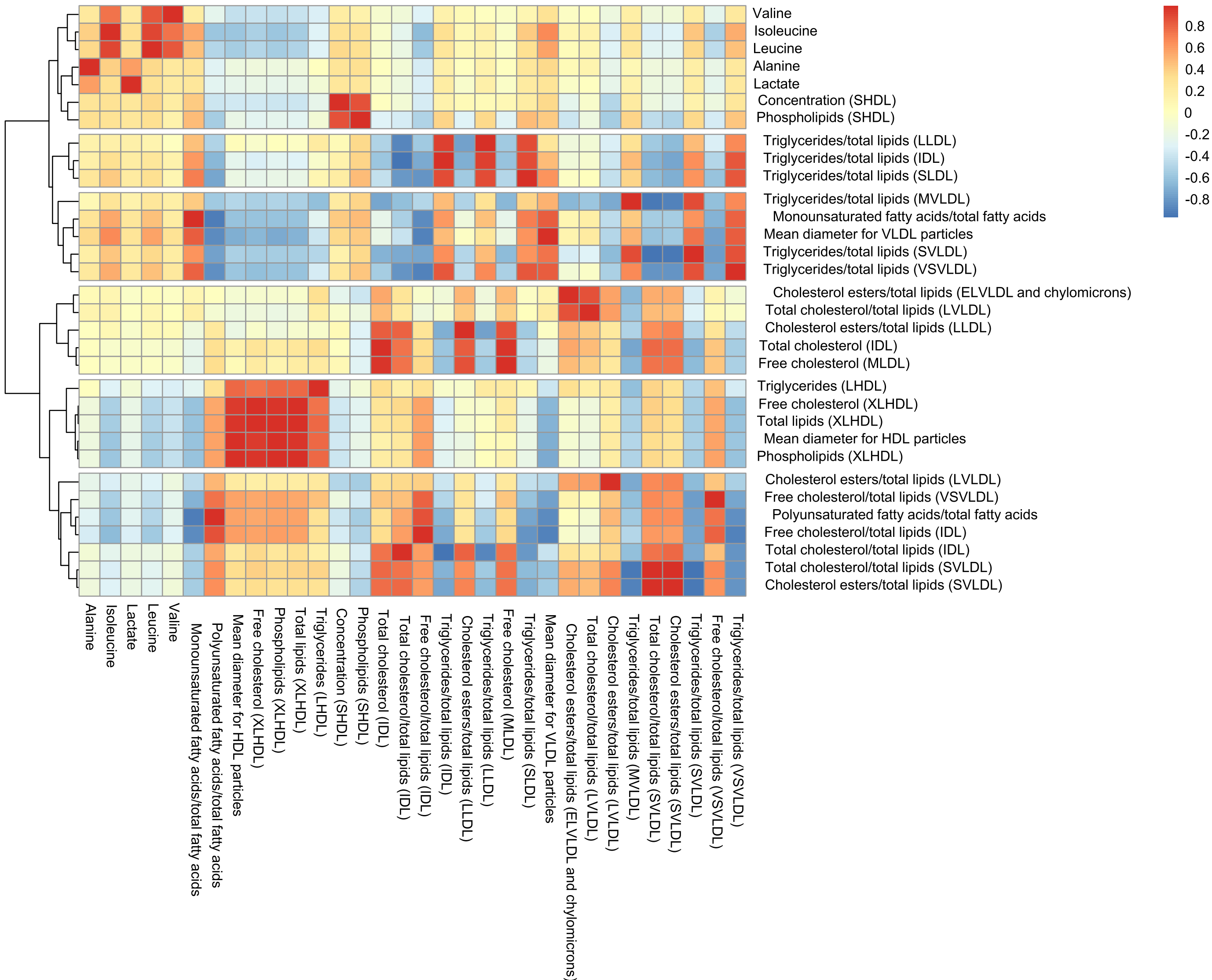

Fig. 2. Correlation matrix among the 32 glycemic profile-related lipid biomarkers.

associations not attaining statistical significance but showing comparable risk estimates and SHAP value (Fig. 1).

3.5. Glycemic profile-related lipid biomarkers associated with CACS

Changes in HDL dimension and composition associated with CACS. As shown in Table 3, HDL diameter was inversely associated with CACS with a multi-adjusted OR of 0.85 and 95 %CI (0.78–0.92). When the analysis was stratified by dispensed lipid lowering drugs, the significance was not attained in the group with a dispensed lipid lowering drug. Consistently, the concentration of small HDL associated with CACS, but statistical significance was borderline when presence of a dispensed lipid lower drug was added to the model. We observed a borderline statistical significant interaction between the concentration of small HDL and dispensed lipid lowering drug with an OR of 0.53 and 95 %CI (0.36–0.77) and between the concentration of small HDL and dispensed statins with an OR of 0.56 and 95 %CI (0.39–0.82), suggesting that lipid lowering drugs were partly reducing the risk of CACS associated with a relatively high concentration of circulating small HDL (Table 4).

Changes in the composition of extra-large HDL associated with CACS. In particular, an inverse association between free cholesterol, phospholipids and total lipids content associated with a lower risk of CACS.

None of the 32 glycemic profile-related lipid biomarkers was associated with relative increase of CACS in study participants with CACS>0 after adjustment for cardiovascular risk factors and dispensed lipid lowering drugs (Supplementary Table X), and no interaction was observed between any of the biomarkers and dispensed lipid lowering drugs or statin on CACS relative increase (Supplementary Table XI).

When the analysis was performed using an imputed dataset, comparable risk estimates were observed, but not all associations attained statistical significance (Supplementary Table XI).

4. Discussion

We identified 32 biomarkers mirroring circulating levels of lipoproteins, lipoprotein components and metabolites, associated with prediabetes and diabetes in a contemporary Swedish population. Among these 32 biomarkers, an excess of small circulating HDL associated with the occurrence of subclinical coronary atherosclerosis, estimated by CACS. Further, we show that, if lipid lowering drugs were dispensed, the association of small HDL with the occurrence of CACS was modified, although the interaction analysis did not attain a formal statistical significance.

Statins have multiple effects on the composition and concentration of

Table 3
Association between each one of the 32 glycemic profile-related lipid biomarkers with CACS in the SCAPIS-OMICS and in study participants with and without a dispensed lipid lowering drug.

	Model 1 (n = 4773)		Model 2 (n = 4598)		Model 3 (n = 4598)		Lipid lowering (n = 627)		Non lipid lowering (n = 4146)	
	OR (95 %CI)	*P*-value	OR (95 %CI)	P-value	OR (95 %CI)	P-value	OR (95 %CI)	P-value	OR (95 %CI)	P-value
Alanine	1.11 (1.05–1.18)	1.33e-03	1.01 (0.95–1.09)	6.73e-01	0.98 (0.92–1.06)	6.46e-01	1.21 (1.02–1.44)	2.60e-02	0.94 (0.87–1.02)	1.32e-01
Isoleucine	1.52 (1.43–1.63)	**<1.00e-05**	1.16 (1.08–1.25)	**<1.00e-05**	1.12 (1.03–1.21)	6.00e-03	1.10 (0.92–1.32)	2.96e-01	1.12 (1.02–1.23)	1.13e-02
Lactate	1.18 (1.11–1.25)	**<1.00e-05**	1.00 (0.93–1.06)	8.98e-01	0.98 (0.92–1.05)	6.09e-01	1.05 (0.88–1.26)	5.95e-01	0.97 (0.90–1.05)	4.83e-01
Leucine	1.54 (1.44–1.65)	**<1.00e-05**	1.18 (1.09–1.27)	**<1.00e-05**	1.13 (1.04–1.22)	1.33e-03	1.09 (0.91–1.29)	3.81e-01	1.14 (1.05–1.26)	2.00e-03
Valine	1.42 (1.33–1.51)	**<1.00e-05**	1.11 (1.03–1.19)	2.67e-03	1.09 (1.01–1.17)	2.73e-02	1.07 (0.88–1.27)	5.11e-01	1.09 (1.01–1.19)	3.27e-02
MUFA/TFA	1.33 (1.25–1.41)	**<1.00e-05**	1.09 (1.01–1.17)	2.13e-02	1.04 (0.97–1.12)	2.68e-01	1.13 (0.94–1.39)	2.02e-01	1.02 (0.94–1.11)	6.03e-01
PUFA/TFA	0.75 (0.71–0.80)	**<1.00e-05**	0.93 (0.86–1.00)	4.87e-02	0.97 (0.90–1.04)	3.81e-01	0.83 (0.69–1.00)	4.60e-02	1.00 (0.92–1.10)	9.11e-01
Diameter HDL	0.63 (0.59–0.67)	**<1.00e-05**	0.82 (0.76–0.89)	**<1.00e-05**	0.85 (0.78–0.92)	**<1.00e-05**	0.88 (0.70–1.12)	2.86e-01	0.84 (0.77–0.92)	**<1.00e-05**
Extra large HDL										
Free Chol	0.64 (0.60–0.69)	**<1.00e-05**	0.84 (0.77–0.92)	**<1.00e-05**	0.87 (0.80–0.95)	**<1.00e-05**	0.88 (0.70–1.13)	3.22e-01	0.87 (0.79–0.95)	2.00e-03
Phospholipids	0.63 (0.59–0.67)	**<1.00e-05**	0.83 (0.76–0.91)	**<1.00e-05**	0.86 (0.79–0.93)	**<1.00e-05**	0.88 (0.70–1.12)	2.80e-01	0.86 (0.78–0.94)	1.33e-03
Total lipids	0.64 (0.60–0.68)	**<1.00e-05**	0.84 (0.77–0.92)	**<1.00e-05**	0.87 (0.80–0.95)	**<1.00e-05**	0.88 (0.70–1.13)	3.03e-01	0.87 (0.79–0.95)	2.00e-03
Large HDL										
Triglycerides	0.72 (0.68–0.77)	**<1.00e-05**	0.92 (0.86–1.00)	3.80e-02	0.95 (0.88–1.02)	1.57e-01	0.94 (0.78–1.15)	5.71e-01	0.95 (0.87–1.03)	1.86e-01
Small HDL										
Concentration	1.32 (1.25–1.41)	**<1.00e-05**	1.17 (1.09–1.26)	**<1.00e-05**	1.14 (1.06–1.22)	1.33e-03	1.13 (0.93–1.37)	2.08e-01	1.14 (1.05–1.23)	**6.67e-04**
Phospholipids	1.19 (1.13–1.27)	**<1.00e-05**	1.10 (1.02–1.18)	1.33e-02	1.05 (0.98–1.13)	1.89e-01	1.14 (0.94–1.36)	1.63e-01	1.03 (0.95–1.11)	4.70e-01
Mean diameter VLDL	1.36 (1.29–1.44)	**<1.00e-05**	1.08 (1.00–1.17)	4.20e-02	1.04 (0.96–1.12)	3.15e-01	1.15 (0.95–1.40)	1.60e-01	1.01 (0.93–1.10)	7.48e-01
Extra-large VLDL and chylomicrons										
CE/total lipids	1.05 (0.99–1.12)	9.87e-02	1.05 (0.98–1.12)	1.93e-01	1.08 (1.01–1.16)	2.47e-02	0.99 (0.83–1.21)	9.44e-01	1.09 (1.02–1.18)	1.93e-02
Large VLDL										
Total Chol/total lipids	1.05 (0.99–1.11)	1.12e-01	1.01 (0.94–1.08)	8.63e-01	1.03 (0.97–1.11)	3.52e-01	0.95 (0.79–1.14)	5.75e-01	1.05 (0.97–1.13)	2.05e-01
CE/total lipids	0.85 (0.80–0.90)	**<1.00e-05**	0.95 (0.88–1.02)	1.24e-01	1.00 (0.93–1.07)	9.46e-01	0.87 (0.71–1.05)	1.61e-01	1.02 (0.95–1.11)	6.05e-01
Medium VLDL										
TG/total lipids	1.15 (1.09–1.22)	**<1.00e-05**	1.00 (0.93–1.07)	9.75e-01	0.95 (0.88–1.02)	1.38e-01	1.07 (0.90–1.28)	4.31e-01	0.92 (0.85–1.00)	3.87e-02
Small VLDL										
Total Chol/total lipids	0.92 (0.87–0.97)	4.00e-03	1.04 (0.97–1.11)	3.38e-01	1.10 (1.02–1.18)	4.00e-03	0.94 (0.80–1.11)	4.70e-01	1.14 (1.06–1.24)	**<1.00e-05**
CE/total lipids	0.93 (0.88–0.99)	1.47e-02	1.03 (0.96–1.10)	3.81e-01	1.10 (1.03–1.18)	4.00e-03	0.95 (0.81–1.11)	4.97e-01	1.14 (1.05–1.24)	**<1.00e-05**
TG/total lipids	1.18 (1.12–1.26)	**<1.00e-05**	0.99 (0.92–1.06)	6.95e-01	0.93 (0.86–0.99)	3.53e-02	1.09 (0.92–1.28)	3.37e-01	0.89 (0.82–0.96)	2.67e-03
Extra-small VLDL										
Free Chol/total lipids	0.77 (0.72–0.82)	**<1.00e-05**	0.94 (0.88–1.01)	1.25e-01	0.97 (0.90–1.05)	5.07e-01	0.89 (0.73–1.05)	1.86e-01	1.00 (0.91–1.09)	9.15e-01
TG/total lipids	1.27 (1.20–1.35)	**<1.00e-05**	1.06 (0.98–1.14)	1.18e-01	0.98 (0.91–1.06)	5.95e-01	1.07 (0.92–1.25)	3.69e-01	0.95 (0.87–1.04)	2.87e-01
IDL										
Total Chol	1.02 (0.96–1.08)	4.93e-01	1.08 (1.01–1.15)	2.13e-02	1.13 (1.06–1.22)	**<1.00e-05**	0.97 (0.83–1.13)	6.77e-01	1.18 (1.09–1.28)	**<1.00e-05**
Total Chol/total lipids	0.91 (0.85–0.96)	1.33e-03	0.98 (0.91–1.05)	5.26e-01	1.06 (0.98–1.14)	1.27e-01	0.96 (0.84–1.10)	5.78e-01	1.09 (1.01–1.19)	3.33e-02
Free Chol/total lipids	0.75 (0.70–0.80)	**<1.00e-05**	0.91 (0.84–0.98)	1.07e-02	0.96 (0.89–1.04)	3.15e-01	0.93 (0.78–1.07)	3.20e-01	0.97 (0.89–1.06)	5.62e-01
TG/total lipids	1.14 (1.08–1.21)	**<1.00e-05**	1.06 (0.99–1.13)	1.19e-01	0.98 (0.91–1.05)	5.57e-01	1.05 (0.92–1.20)	5.13e-01	0.96 (0.88–1.04)	3.17e-01
Large LDL										
CE/total lipids	1.12 (1.05–1.19)	**<1.00e-05**	1.07 (1.00–1.14)	5.67e-02	1.13 (1.06–1.21)	**<1.00e-05**	0.99 (0.85–1.14)	9.11e-01	1.18 (1.09–1.28)	**<1.00e-05**

(*continued on next page*)

Table 3 (*continued*)

	Model 1 (*n* = 4773)		Model 2 (*n* = 4598)		Model 3 (n = 4598)		Lipid lowering (*n* = 627)		Non lipid lowering (*n* = 4146)	
	OR (95 %CI)	*P*-value	OR (95 %CI)	P-value	OR (95 %CI)	P-value	OR (95 %CI)	P-value	OR (95 %CI)	P-value
TG/total lipids	1.02 (0.96–1.08)	5.46e-01	1.03 (0.96–1.10)	4.03e-01	0.97 (0.90–1.04)	3.32e-01	1.05 (0.91–1.20)	5.23e-01	0.94 (0.87–1.02)	1.57e-01
Medium LDL										
Free Cholesterol	1.06 (1.00–1.12)	6.13e-02	1.09 (1.02–1.16)	1.13e-02	1.13 (1.06–1.22)	**<1.00e-05**	0.96 (0.82–1.11)	5.66e-01	1.18 (1.10–1.28)	**<1.00e-05**
Small LDL										
TG/total lipids	1.11 (1.05–1.18)	**<1.00e-05**	1.04 (0.97–1.10)	3.05e-01	0.98 (0.91–1.05)	5.85e-01	1.06 (0.92–1.25)	4.30e-01	0.96 (0.88–1.04)	3.11e-01

Model 1: univariable analysis.
Model 2: adjusted by age- sex- BMI- smoking- alcohol consumption- PA- SES- SBP- DBP;
Model 3: model 2+ dispensed lipid lowering drugs.
Missing values as in Table 1.
CACS is defined as presence of coronary artery calcium if >0 according to Agatston criteria [14].
In bold- significant association with a *p value* ≤ 0.001312.
Abbreviations: CE: cholesterol esters; FA: Fatty Acids; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA; TG: triglycerides; TFA: Total Fatty Acid.

lipoproteins. and are strongly recommended in individuals with diabetes to reduce the risk of cardiovascular events [1]. To the best of our knowledge, this is the first systematic analysis of the interactions between lipid lowering drugs and circulating lipoproteins. Our data suggest that lipid lowering drugs may reduce up to 40 % the occurrence of CACS associated with high circulating levels of small HDL. While most of the literature has focused on the association of lipid metabolites with the risk of incident type 2 diabetes [21–24], our study shows that changes in lipid, lipoproteins and lipoprotein lipid content are present already in individuals with pre-diabetes thus representing a marker of cardiovascular risk before the onset of overt diabetes.

The occurrence of coronary artery calcium was associated with HDL size, concentration, and composition. Consistently with previous observations, in normoglycemic study participants circulating levels of small HDL were slightly increased in those treated with statins as compared to non treated [25,26], which we also observed in the group with pre-diabetes. Small HDL associated with prevalent coronary heart disease in the Framingham Offspring Study [27], with the risk of myocardial infarction [21] and ischemic stroke in a nested case control study from the China Kadoorie Biobank [28], and with mortality risk in patients with known coronary heart disease [29]. Former studies implicate changes in the cholesterol efflux regulation as a potential mechanism linking HDL to cardiovascular risk [30–32] and different HDL functional properties [33] associated with both diabetes and coronary heart disease [32]. In this latter study, there were no common metabolites between diabetes and coronary heart disease, although the study population was probably too small to detect them [32]. None of the small metabolites, VLDL, IDL, or LDL concentration or composition associated with CACS. However, as lipoproteins exchange cholesterol among each other and with peripheral tissues [34], we cannot exclude that the observed association of HDL with occurrence of CACS is secondary to changes in the metabolism/composition of non-HDL lipoprotein.

The composition of lipoproteins is highly dynamic and directly correlated with the circulating lipid levels, insulin resistance, and glycemic control [35]. Our study is based on the results of NMR which allows the evaluation of specific patterns and relative changes in lipid metabolites and lipoprotein composition. The mechanisms underlying the pattern of lipoproteins in pre-diabetes and diabetes are largely secondary to insulin resistance. Insulin resistance causes an increased flux of fatty acids (we observed a relative excess of MUFA in individuals with diabetes) and an increased triglycerides production in the liver which results in larger and more triglyceride rich VLDL. The lipoprotein-insulin resistance score was developed to identify individuals at high risk of developing diabetes. Among all lipoproteins, VLDL size and number of particles showed the highest weight in the score [36]. Consistently, we observed larger VLDL in individuals with pre-diabetes and diabetes, but also a relative increase in triglycerides concentration in VLDL, IDL and LDL regardless of concentration and dimension of the single lipoprotein.

Diabetes associated dyslipidemia is commonly defined by the presence of high triglycerides, low HDL, and elevated levels of small dense LDL. Total- and LDL-cholesterol levels are commonly reported as normal or slightly higher, as compared to the normoglycemic population [2]. In our study, we observed that total as well as LDL-cholesterol levels were consistently lower in pre-diabetes and diabetes than in normoglycemia in the presence and absence of dispensed lipid lowering drugs. Lower LDL-cholesterol levels have been reported in individuals with diabetes in both observational [9] and interventional studies [5] with low LDL-cholesterol levels associated with an increased risk of coronary events in the presence of high triglycerides and low HDL levels [37]. Recently, biomarkers associated with LDL-cholesterol were found to be inversely related to the risk of developing diabetes in the UK Biobank [24]. Of note, Mendelian randomization studies have shown that a polymorphism associated with a lower activity of the HMG-CoA-reductase, the key enzyme in cholesterol synthesis targeted by statins, associated with the risk of diabetes [38]. Low LDL-cholesterol may precede the onset of clinically overt diabetes and, as previously reported, identify a group at higher risk of cardiovascular events [37]. The mechanisms by which statins may increase the risk of developing diabetes are not fully understood [39], but the protective effect against cardiovascular events outweighs the risk associated with diabetes [40].

4.1. Limitations

Our study has several strengths and limitations. The strengths are related to the well characterized study population with a granularity of molecular, imaging, and clinical data representative of a contemporary population of individuals with pre-diabetes and diabetes. We used a very conservative threshold for attaining statistical significance in this analysis, the main reason being that to advance in knowledge in precision medicine we need to reduce the proportion of false positive to increase the likelihood to understand and identify culprit molecules. The main limitations are related to the lack of data on the enzyme activity of crucial mediators of lipoprotein metabolism whose decreased/increased activity can improve the understanding of the observed pattern in HDL and non-HDL lipoprotein and of other lipoproteins such as Lp(a) and to the cross-sectional study design which hampers inference on causality or temporality of the observed associations. Fifteen study participants had reported onset of diabetes before the age of 40 years and only insulin treatment, making the diagnosis of diabetes type 1 very likely in this

Table 4
Analysis of the interaction between each one of the 32-glycemic profile-related lipid biomarkers and dispensed lipid lowering drug or dispensed statin on the occurrence of CACS.

	Lipid lowering (n = 626)		Statin (n = 607)	
	Interaction	P value	Interaction	P value
Alanine	1.00 (0.71–1.45)	0.99	1.05 (0.74–1.55)	0.79
Isoleucine	1.07 (0.77–1.55)	0.70	1.08 (0.76–1.55)	0.69
Lactate	1.12 (0.79–1.66)	0.53	1.05 (0.72–1.58)	0.80
Leucine	1.01 (0.73–1.45)	0.93	1.07 (0.76–1.52)	0.71
Valine	1.00 (0.69–1.45)	0.98	1.03 (0.71–1.50)	0.88
MUFA/TFA	1.08 (0.75–1.52)	0.67	1.11 (0.76–1.60)	0.59
PUFA/TFA	0.96 (0.68–1.37)	0.80	0.96 (0.66–1.40)	0.81
Mean diameter HDL	0.91 (0.61–1.37)	0.64	0.96 (0.63–1.48)	0.85
Extra Large HDL				
Free Chol	0.91 (0.64–1.32)	0.62	0.92 (0.60–1.43)	0.69
Phospholipids	0.87 (0.57–1.31)	0.51	0.90 (0.58–1.40)	0.62
Total lipids	0.88 (0.58–1.32)	0.54	0.91 (0.59–1.41)	0.66
Large HDL				
TG	1.10 (0.68–1.67)	0.68	0.98 (0.68–1.43)	0.92
Small HDL				
Concentration	0.53 (0.36–0.77)	0.001	0.56 (0.39–0.82)	0.005
Phospholipids	0.64 (0.44–0.91)	0.017	0.69 (0.48–0.98)	0.04
VLDL				
Mean diameter	1.23 (0.85–1.81)	0.24	1.24 (0.86–1.82)	0.26
Extra largeVLDL and CM				
CE/total lipids	1.00 (0.68–1.48)	0.99	1.06 (0.71–1.56)	0.77
Large VLDL				
Total Chol/total lipids	1.02 (0.66–1.50)	0.91	1.06 (0.69–1.58)	0.74
CE/total lipids	1.51 (1.03–2.21)	0.04	1.53 (1.02–2.26)	0.04
Medium VLDL				
TG/total lipids	0.89 (0.63–1.27)	0.49	0.84 (0.60–1.21)	0.34
Small VLDL				
Total Chol/total lipids	0.98 (0.70–1.33)	0.88	1.02 (0.72–1.40)	0.91
CE/total lipids	1.05 (0.75–1.44)	0.76	1.09 (0.78–1.48)	0.62
TG/total lipids	1.16 (0.85–1.63)	0.37	1.11 (0.80–1.59)	0.53
Extra small VLDL				
Free Chol/total lipids	0.75 (0.53–1.05)	0.09	0.76 (0.54–1.08)	0.11
TG/total lipids	1.14 (0.84–1.54)	0.41	1.13 (0.83–1.56)	0.42
IDL				
Total Chol	0.85 (0.62–1.19)	0.37	0.88 (0.63–1.22)	0.44
Total Chol/total lipids	1.02 (0.77–1.36)	0.85	1.03 (0.77–1.37)	0.82
Free Chol/total lipids	0.84 (0.59–1.14)	0.26	0.82 (0.59–1.12)	0.22
TG/total lipids	0.96 (0.73–1.27)	0.78	0.97 (0.74–1.30)	0.82
Large LDL				
CE/total lipids	1.06 (0.78–1.42)	0.68	1.05 (0.77–1.42)	0.75
TG/total lipids	0.88 (0.66–1.17)	0.37	0.89 (0.67–1.21)	0.46
Medium LDL				
Free Chol	0.85 (0.62–1.19)	0.35	0.87 (0.62–1.20)	0.38
Small LDL				
TG/total lipids	1.00 (0.73–1.32)	0.99	1.02 (0.75–1.38)	0.86

Interaction effect is adjusted by age- sex- SES- BMI- moderate and vigorous physical activity- smoking (current vs ex- or non- smoker)- alcohol use- SBP- and DBP (as in model 2). Interaction effect refers to the interaction (odds ratio) between prescription of lipid lowering drugs and each metabolite and prescription of statins categorized as low and high dose and each lipoprotein or metabolite.
Abbreviations: CE: cholesterol esters; Chol: Cholesterol; FA: Fatty Acids; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA TG: triglycerides; TFA: Total Fatty Acid.

group. However, in SCAPIS, no specific diagnostic tests were performed to diagnose type 1 diabetes [41]. As metabolic differences exist between type 1 and type 2 diabetes, we cannot exclude that including this group might have marginally affected the risk estimates. Lipid and glucose levels were measured in all study participants at different study sites using different analytical methods and by NMR at the same site. This results in differences in the absolute value across centers and between methods. However, we observed a high correlation between biochemical and NMR measurements, consistently with a previous report [28]. The NMR panel used in the present analysis classifies the lipoprotein dimension using a different scale from the clinical biochemical classification, therefore caution should be used in comparing our results with studies using different methods to estimate circulating levels of lipoproteins subtypes [42]. Furthermore, as we use a cross-sectional study design, we cannot infer the causality of the observed relationships, which is necessary for implementation in clinical practice. At the same time, our results highlight the complexity of changes in lipid metabolism in prediabetes and diabetes and the need for in-depth phenotyping to improve the treatment and prevention of cardiovascular disease in patients with diabetes. Of note, circulating lipid and lipoproteins levels are influenced by a large number of drugs including those used to treat diabetes, making drug interaction an important residual confounder in our analysis [43,44]. Finally, we lack an external validation of our results.

5. Conclusions

We report the metabolomic landscape in a contemporary population, identify the composition and concentration of small HDL as an important link between dysglycemia and CACS and highlight the potential role of lipid lowering drugs in modifying this association.

CRediT authorship contribution statement

Bruna Gigante: Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Qiaosen Chen:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Harry Björkbacka:** Writing – review & editing, Methodology, Data curation. **Elias Björnson:** Writing – review & editing, Methodology, Formal analysis. **Jonas Brinck:** Writing – review & editing, Visualization, Methodology, Data curation. **Elin Chorell:** Writing – review & editing, Methodology, Data curation. **Demir Djekic:** Writing – review & editing, Methodology, Data curation. **Andreas Edsfeldt:** Writing – review & editing, Investigation, Data curation. **Gunnar Engström:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Jan W. Eriksson:** Writing – review & editing, Methodology, Data curation. **Anders Gummesson:** Writing – review & editing, Methodology, Investigation. **Emil Hagström:** Writing – review & editing, Methodology, Data curation. **Ulf Hedin:** Writing – review & editing, Methodology. **Tomas Jernberg:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Nina Johnston:** Writing – review & editing, Methodology. **Lennart Nilsson:** Writing – review & editing, Data curation. **Fredrik Nyström:** Writing – review & editing, Data curation, Conceptualization. **Julia Otten:** Writing – review & editing, Investigation. **Annika Rosengren:** Writing – review & editing, Investigation, Funding acquisition, Data curation. **Stefan Söderberg:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Jenny Theorell Haglöw:** Writing – review & editing, Methodology. **Carl Johan Östgren:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2024.132698.

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