Metabolomics (2025) 21:57
https://doi.org/10.1007/s11306-025-02253-z

ORIGINAL ARTICLE

Coronary artery disease is associated with particular change of serum metabolome: a case–control study

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Received: 1 July 2024 / Accepted: 2 April 2025

Abstract

Introduction Cardiovascular disease (CVD) is a significant cause of mortality worldwide. Preventive programs are trying to reduce the burden of the disease. Recent advances in metabolomics profiling open a new avenue for developing complementary CVD evaluation strategies.

Objectives The aim of the study was to investigate whether a metabolomic profile can provide an additional characterisation of individuals with coronary artery disease (CAD).

Methods The study included 167 participants with CAD aged 41–79 years. A control group was formed of 166 individuals without CAD, gender- and age-matched to the study group. A total of 188 metabolites were profiled in serum by liquid chromatography-tandem mass spectrometry. After clearing the data, associations between 132 metabolites and CAD presence were analysed using multiple linear regression models.

Results We observed significant differences in serum metabolic profiles between analysed groups on various levels. However, a deeper analysis revealed **sphingomyelin** 41:1 (SM 41:1) as the main metabolite independently associated with CAD after correction for classical CV risk factors. Its concentration was lower in the CAD group (median 9.79 µmol/L, interquartile range (IQR) 7.92–12.23) compared to control one (median 13.60 µmol/L, IQR 11.30–16.15) ($p<0.001$). Further analysis showed that SM 41:1 concentration was inversely correlated with CAD, current smoking, and hypertension; and positively associated with female gender and non-HDL level.

Conclusions CAD patients present lower plasma concentrations of SM 41:1 than healthy subjects. A better understanding of the biological function of sphingomyelin in CAD patients may help develop therapeutic approaches and risk stratification in this group.

Keywords Metabolomics · Cardiovascular disease · Coronary artery disease · **Sphingomyelin** 41:1 · Principal component analysis

1 Introduction

Cardiovascular disease (CVD) is a significant cause of mortality worldwide (Wilkins et al., 2017) as well as in Poland (Pikala & Maniecka-Bryla, 2017). Among these diseases, coronary artery disease (CAD) is the most common cause, accounting for more than 40% of CVD deaths (Pinaire et al., 2019). Epidemiological studies have identified a number of risk factors and disease processes associated with CVD, including hypertension, hyperlipidemia, cigarette smoking, and diabetes mellitus (Dawber et al., 1957). Preventive programs focused on these risk factors are trying to reduce the burden of the disease at the community level

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Published online: 26 April 2025

(Mahmood et al., 2014). Clinically used biomarkers, such as lipid profiles or blood glucose levels, reflect established traditional risk factors of CAD, but there is still a lack of data regarding biomarkers that reflect risks not captured by traditional risk factors. Biomarkers such as cardiac troponin have been identified as predictors of adverse CVD events (Wang, 2011). However, these established biomarkers are usually detected after myocardial injury. Earlier detection of biomarkers in plasma and identification of molecules specifically associated with CVD events is important for prognostic evaluation in patients with CAD.

Metabolomics provides a promising and effective approach to uncover metabolic abnormalities associated with various pathological conditions (Johnson et al., 2016; Lv et al., 2024; Marcinkiewicz-Siemion et al., 2018). It offers a comprehensive picture of metabolic changes during disease, with significant potential to identify circulating metabolite biomarkers in patients with CAD. Variations in the metabolome have been associated with the presence and severity of CAD, pointing to its potential role as a clinical biomarker (Vernon et al., 2021). Metabolomic profiles have been shown to predict individuals' metabolic responses to dietary interventions, emphasizing the need for a personalized approach to CVD risk management (Adamska-Patruno et al., 2019). In addition, several studies have found that metabolomics profiling can forecast future CVD risk independently of traditional risk factors, thereby offering an invaluable complementary tool for risk stratification (Li et al., 2020). Given the significant heterogeneity of the mechanisms underlying CAD, the extensive information contained in metabolomic profiles has yet to be fully explored for CVD risk prediction. Elucidating metabolic disorders could potentially identify biomarkers for CAD, as well as improve our understanding of the molecular processes involved in various outcomes.

The aim of the study was to investigate whether a metabolic profile can provide an additional characterisation of individuals with CAD. In this study, we performed a case–control analysis of patients with CAD to examine their serum metabolic profile compared to individuals without cardiovascular events.

2 Patients and methods

2.1 Study population

The study followed the guidelines outlined in the Strengthening the reporting of observational studies in epidemiology (STROBE) (Supplementary material 1).

Based on medical records from three cardiology departments with catheterization laboratory (Bialystok, Poland), all patients hospitalised for 12–26 months due to acute coronary syndrome (ST-segment elevation acute myocardial infarction (STEMI), non-ST-segment elevation acute myocardial infarction (NSTEMI), unstable angina/acute myocardial ischaemia) or planned percutaneous coronary intervention (PCI) at the beginning of the study (n = 349). Following a telephone contact and invitation, 257 individuals were surveyed. Active cancer was an exclusion criterion. Patients with active infectious diseases were not examined; therefore, their appointment at the study center was postponed until they recovered. The metabolomic profile was determined in 170 randomly selected individuals. Three individuals had missing data from the interview and were excluded from further analysis. Finally, the study group included 167 patients with stable CAD aged 41–79 years.

The control group of 166 individuals without CAD (based on interview or medical history), gender- and age-matched to the CAD patients, was selected. The control group was recruited from the Bialystok PLUS study (Poland). The Bialystok PLUS study design has already been described (Chlabicz et al., 2023). In brief, participants were randomly selected to reflect the city's demographic structure. Annually, a pseudonymised list of citizens aged 20–79 was provided by the Municipal Office in Bialystok, categorized by sex and 5-year age intervals. From each subcategory, random samples were drawn to ensure a proportional representation matching the city's population. There were no exclusions, only certain restrictions. Participants who had an acute infectious disease or had undergone surgery within the previous six weeks were not examined and were asked to return to the study after this period.

The selection of study groups is shown in the flow chart (Fig. 1).

All patients in both the CAD and control groups underwent a detailed health evaluation at the same research centre and with the same standard operating procedures. Details of the methods were described previously (Chlabicz et al., 2022, 2023; Sawicka-Smiarowska et al., 2021). In brief, patient-level information was collected using a structured data collection form at the time of recruitment. Collected baseline data included demographics, anthropometric measures, past medical history, and current medication use.

Fasting blood samples were taken at the time of recruitment and stored in a biobank. A list of parameters with the method and equipment used is presented in Supplementary material 2, Table S1.

Anthropometric measurements including height, weight, as well as waist and hips circumferences were taken. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. BMI classifications ware: normal weight (to 24.9), overweight (25 to 29.9), and obese (30 or more). Waist-to-hip ratio (WHR)

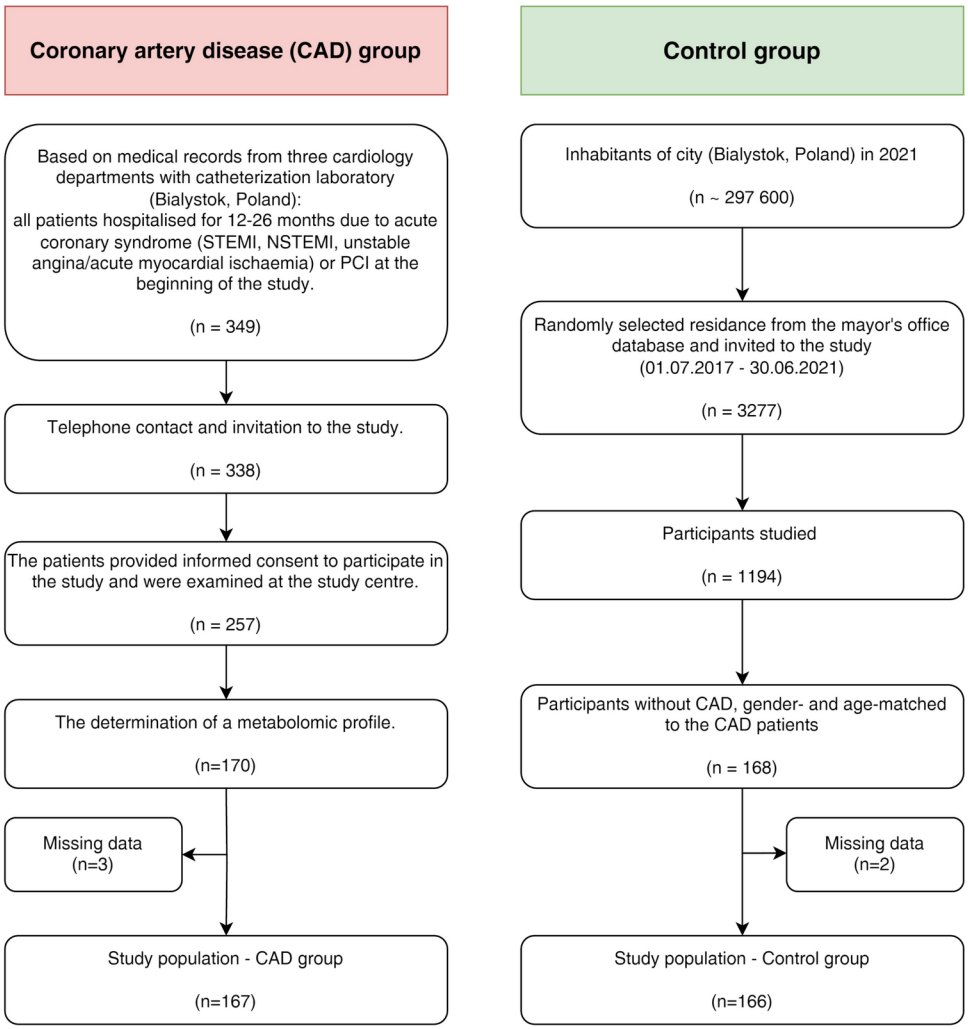

Fig. 1 Study flow diagram

was calculated as a ratio between waist and hips circumference. The echocardiography (ECHO) measurements were made using the B-mode ultrasound. In ECHO, measurements of the dimensions of the heart, left atrial (LA) volume, left ventricular ejection fraction (LVEF) using the Biplane method were performed. The left ventricular mass (LVM) was calculated using the formula $LVM = 0.8\ (1.04\ (IVST + LVID + LPWT)^3 - (LVID)^3 + 0.6)$, where IVST is interventricular septal thickness, LVID is left ventricular internal dimension, and LPWT is left ventricle posterior wall thickness. Body surface area (BSA) was calculated by the formula: $BSA = (W - 60) \times 0.01 + H$, where BSA is the body surface area in squared meters, W is the weight in kilograms, and H is the height in meters. The left ventricular mass index (LVMI) was calculated by the formula LVM/BSA ($LVMI_{BSA}$), then the left ventricular hypertrophy (LVH) was defined as $LVMI \geq 115\ g/m^2$ for men and $\geq 95\ g/m^2$ for women. The left atrial volume index (LAVI) was calculated by dividing left atrial volume by BSA; enlarged volume was defined as $> 34\ ml/m^2$.

2.2 Quantitation of metabolites

Targeted metabolomics using the high-throughput liquid chromatography-tandem mass spectrometry technique was performed for serum samples collected from all study participants. In total, 188 metabolites were measured using an AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). Sample preparation and measurements were performed according to the protocol and methodology provided by the manufacturer (Sawicka-Smiarowska et al., 2021). Analyses were performed using an LC–MS/MS system consisting of a 1290 UHPLC coupled to a 6470A Triple Quad mass spectrometer (both Agilent Technologies, Santa Clara, California, USA). The processing, quantification, and normalisation of raw spectral data were carried out utilising the MetIDQ software (Oxygen DB110-3005, Biocrates, Life Science AG, Innsbruck, Austria). The data normalisation and cleaning were performed according to the guidelines outlined in the Biocrates' kit user manual (Godlewski et al., 2023). Data cleaning included the removal of metabolites

with low reproducibility assessed based on the coefficient of variation (CV) calculated from triplicates of the quality control (QC) samples for each metabolite in each batch, where the rejection criterion was a coefficient of variation value above 30%. Then, the obtained data were filtered to remove metabolites below the detection limit (LOD) in more than 20% of the samples. For the remaining metabolites, values below the LOD were replaced by ½ of the detection limit for each metabolite in each batch (Godlewski et al., 2023). A total of 132 metabolites were included for further analysis. A full list of metabolites included for statistical analysis, together with their CVs (median value 6.55%, IQR 5.57–7.99), can be found in Table S2.

2.3 Statistical analysis

Categorical variables are displayed as frequency distributions (n) and percentages (%). The normality of continuous data was assessed using the Shapiro–Wilk test. Since most variables had distributions significantly different from normal, a non-parametric test (the Mann–Whitney *U* tests) was used. Consequently, the results are presented as medians and interquartile ranges (IQR). The chi2 test was used for the univariate comparison between the groups.

Associations between CAD presence and metabolites were analysed using multiple linear regression models. Multiple regression models were adjusted for body mass index (BMI), glomerular filtration rate (GFR), diabetes—Model 1 or BMI, GFR, diabetes, smoking status, non-HDL cholesterol, alanine aminotransferase, *high-sensitivity C-reactive protein* (hsCRP) and statin therapy—Model 2. Regression models were presented using unstandardised and standardised variable coefficients, *p*-values of Wald tests and adjusted coefficients of determination for the model (R^2). Bonferroni's correction was applied in the analyses when associations for multiple metabolites were evaluated simultaneously. This allowed the identification of metabolites that differed significantly between the study groups.

Next, we assessed what parameters were related to the concentration of the identified metabolites. Spearman's non-parametric correlation was used to assess the association of **metabolites** with quantitative variables. Comparisons of metabolites within subgroups by qualitative features were made using the Mann–Whitney U-test. Multiple linear regression models were adjusted for age and sex (Model 1) or age, sex, BMI, systolic blood pressure (BPs), hypertension, diabetes, smoking status, non-HDL cholesterol, hemoglobin A1c (HbA1c), and statin dose equivalent to simvastatin (Model 2) for CAD group and control group. The final model for the entire study population (Model 3) was adjusted for age, sex, BMI, BPs, hypertension, diabetes, smoking status, non-HDL cholesterol, HbA1c, and statin dose equivalent to simvastatin.

The metabolites concentrations were also explored by use of principal component analysis (PCA), an unsupervised multivariate statistical tool used to analyse dataset consisting of a large number of variables. It reduces multidimensional data into principal components that can be viewed by using low-dimensional plots. The Kaiser rule was used to extract the most relevant PCA factors with eigenvalues above 1. The Varimax method was implemented to rotate the principal component matrix. Analysis of variance (ANOVA) has been used to assess the ability of factors to differentiate between CAD and healthy groups, and ANOVA F statistic values were used to identify the most relevant factors.

The statistical analyses were performed using IBM SPSS Statistics 27.0 software (SPSS, Armonk, NY, USA). Statistical hypotheses were verified at a 0.05 significance level.

3 Results

3.1 Characteristics of the study population and control group

The study population were at a median age of 62 years (IQR 58–66), of whom 31.2% (n = 53) were female, 68.8% (n = 117) were male, 23.4% were current smokers and 39.6% were diagnosed with diabetes mellitus. The control group were at a median age of 62 years (IQR 53–66), of whom 38.7% (n = 65) were female, 61.3% (n = 103) were male, 18.1% were current smokers, and 21% were diagnosed with diabetes mellitus. The study and control groups did not differ in age (p = 0.096), sex (p = 0.147), or smoking status (p = 0.235). In contrast, more people in the study group had diabetes mellitus (p < 0.001). The study population is presented in Table 1.

3.2 CAD presence and metabolite profile

We observed differences in the serum metabolomic profiles between CAD patients and a general population (Bialystok PLUS cohort) without CAD. The dependent variable was group belonging. Of the 132 metabolites selected for statistical analysis, 67 (50.8%) were significantly different between the groups (Table S3). Furthermore, 52 metabolites were significantly different (39.4%) in multiple linear regression models adjusted for BMI, GFR, and diabetes (Model 1). After adjusting for BMI, GFR, diabetes, smoking status, non-HDL cholesterol, alanine aminotransferase, hsCRP, statin therapy (Model 2) only one metabolite remained significant after Bonferroni correction—**sphingomyelin** 41:1 (SM 41:1) ($p < 0.001$, standardised ß coefficient

Table 1 The study population

Characteristics	CAD group n = 167	Control group n = 166	*p*
Age, years; median (IQR)	62.0 (58–66)	62.0 (53–66)	0.096
BPs, mmHg; median (IQR)	130.5 (122–143)	127.8 (117–143)	0.239
BPd, mmHg; median (IQR)	83.5 (77–91)	83.0 (77–91)	0.973
HR, bpm; median (IQR)	65.5 (59–73)	71.0 (64–80)	< 0.001
Height, cm; median (IQR)	170.5 (162–177)	170.8 (162–176)	0.433
Weight, kg; median (IQR)	86.0 (74–98)	81.6 (72–93)	0.078
BMI, kg/m^2; median (IQR)	29.5 (27–33)	28.4 (25–32)	0.007
Waist circumference, cm; median (IQR)	100.5 (90–106)	94.0 (84–103)	< 0.001
Hip circumference, cm; median (IQR)	103.0 (97–111)	102.0 (96–107)	0.072
WHR; median (IQR)	0.96 (0.9–1.0)	0.92 (0.8–1.0)	0.001
Fasting glucose, mg/dL; median (IQR)	105.0 (97–120)	101 (93–109)	0.004
OGTT 120 min glucose, mg/dL; median (IQR)	130.0 (100–161)	129 (104–171)	0.597
HbA1c, %; median (IQR)	5.8 (6–6)	5.7 (5–6)	< 0.001
TC, mg/dL; median (IQR)	154.5 (131–184)	195.5 (168–228)	< 0.001
LDL-c, mg/dL; median (IQR)	85.1 (69–109)	134.5 (104–161)	< 0.001
HDL-c, mg/dL; median (IQR)	48.0 (41–60)	57.0 (45–69)	< 0.001
TG, mg/dL; median (IQR)	114.0 (77–167)	114.0 (82–151)	0.968
non-HDL, mg/dL; median (IQR)	101.5 (81–133)	140.7 (113–172)	< 0.001
Creatinine, µmol/L; median (IQR)	83.5 (73–97)	75.7 (65–84)	< 0.001
eGFR, mL/min/1.73m^2; median (IQR)	78.3 (69–94)	88.7 (78–100)	< 0.001
hsCRP, mg/L; median (IQR)	1.0 (0.6–2.0)	0.9 (0.5–2)	0.195
Current smokers; n (%)	39 (23.4)	30 (18.1)	0.235
Hypertension history; n (%)	133 (79.6)	76 (45.8)	< 0.001
Hypercholesterolemia history; n (%)	131 (78.4)	80 (48.2)	< 0.001
Diabetes[a]; n (%)	65 (38.9)	35 (21.1)	< 0.001
Statin therapy; n (%)	150 (89.8)	37 (22.3)	< 0.001
Hypertension therapy; n (%)	161 (96.4)	83 (50)	< 0.001

[a]History or fasting glucose ≥ 126 mg/dL or OGGT 120 min glucose ≥ 200 mg/dL or HbA1c ≥ 6.5%

BPs systolic blood pressure, *BPd* diastolic blood pressure, *bpm* beats per min, *BMI* body mass index, *hsCRP* high-sensitivity C-reactive protein, *eGFR* estimated glomerular filtration rate, *HbA1c* hemoglobin A1c, *HDL-c* high-density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance, *HR* heart rate, *LDL-c* low-density lipoprotein, *mmHg* millimetres of mercury, *OGTT* oral glucose tolerance test, *TC* total cholesterol, *TG* triglycerides, *WHR* waist-hip ratio

− 0.216) (Fig. 2). A high-resolution CID spectrum of SM 41:1 obtained in the positive ion mode using Q-TOF mass spectrometer is included in the supplementary material file (Fig. S1). The analysis revealed that the CAD group had significantly lower SM 41:1 (median 9.79 µmol/L, IQR 7.92, 12.23) concentrations compared to the control group (median 13.60 µmol/L, IQR 11.30–16.15) despite correction of known cardiovascular risk factors. The Full dataset is provided in Tables S3 and S4 and shown in Fig. 2.

3.3 SM 41:1 and clinical, echocardiographic, and laboratory parameters

Correlation was used to assess the association of SM 41:1 concentration with selected clinical, echocardiographic, and laboratory parameters. It was found to be positively correlated with the female gender within both groups (Table S5). A multiple linear regression model was adjusted for age and sex (Model 1), which showed an inverse relationship between SM 41:1 and history of hypertension and LAVI in the control group, a positive correlation with total cholesterol, LDL, TG, and non-HDL in both study groups. In addition, in the control group, a positive correlation with a history of hypercholesterolemia was observed. In contrast, there was no association with age, anthropometric parameters, diabetes, kidney, or liver function. The precise data are provided in Tables S6, S7, S8 and shown in Fig. 3.

Multiple linear regression Model 2 used age, sex, BMI, BPs, hypertension, diabetes, smoking status, non-HDL cholesterol, HbA1c, statin dose equivalent to simvastatin showed a positive correlation of SM 41:1 with female gender and non-HDL independently in both groups, inverse correlation with hypertension in control group, and inverse correlation with current smoking in CAD group (Table S9, Fig. 4 Panel A). The final Model 3 was created for the entire study population and included the following variables: study group, age, sex, BMI, BPs, hypertension, diabetes, smoking status, non-HDL cholesterol, HbA1c, and statin dose equivalent to simvastatin. After accounting for the abovementioned parameters, SM 41:1 level was inversely associated with CAD group, current smoking, and hypertension, while positively with female sex and non-HDL levels. In addition, we showed that the association of SM 41:1 concentration with cholesterol level was independent of statin use and statin dose (Table S10, Fig. 4 Panel B).

3.4 PCA analysis

The metabolites concentrations were investigated using PCA. Twenty principal components (PC) with eigenvalues > 1 were extracted from all of the data, accounting for 84.1% of the variance in the dataset. We assessed how

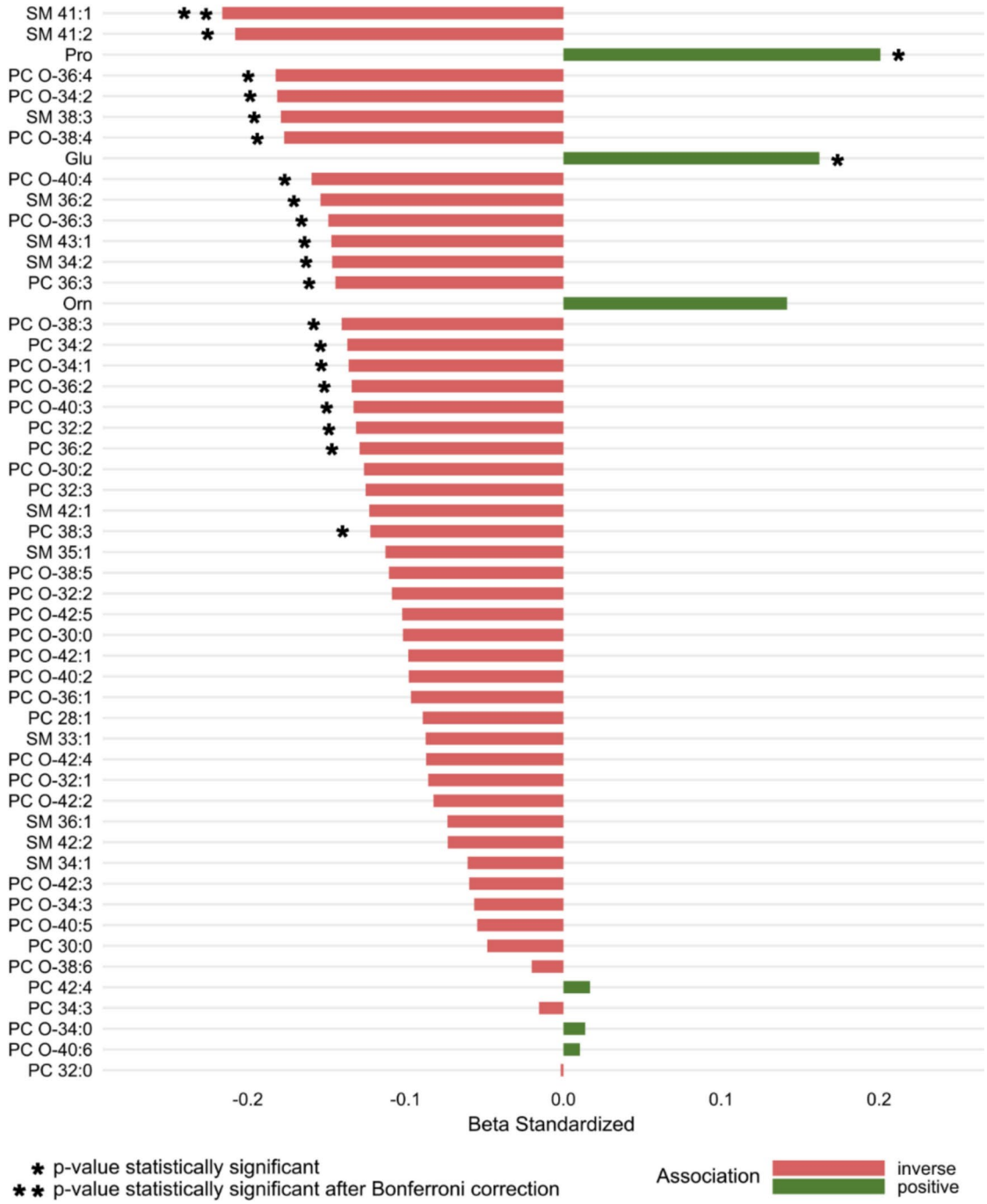

Fig. 2 Metabolites significantly associated with CAD in multiple linear regression models adjusted for BMI, GFR, and diabetes (Model 1) and its association with CAD in Model 2 (adjusted for BMI, GFR, diabetes, smoking status, non-HDL cholesterol, alanine aminotransferase, hsCRP, statin therapy)

extracted factors differentiate the CAD and control groups. It was found that PC 2 and PC 7 differentiated these groups the most. The results indicate that PC 2 and PC 7 may distinguish individuals with CAD from healthy subjects. The precise data are provided in Table S11, S12 and shown in Fig. 5. Highest loadings in PC 2 had: SM 41.1 (0.86), SM 41:2 (0.85), SM 35:1 (0.79), SM 36:2 (0.74), PC 28:1 (0.74), SM 34:1 (0.73), SM 34:2 (0.72), SM 38:3 (0.7), PC O-36:2 (0.69), PC O-40:3 (0.68), SM 36:1 (0.65), SM 33:1 (0.63), PC O-34:1 (0.63), PC 32:3 (0.63), PC O-36:1 (0.63), PC 34:2 (0.62), PC 36:2 (0.61) and in PC 7: PC O-36:4 (0.82), PC O-36:5 (0.71), PC O-38:5 (0.71), PC O-36:3 (0.64), PC O-34:2 (0.61).

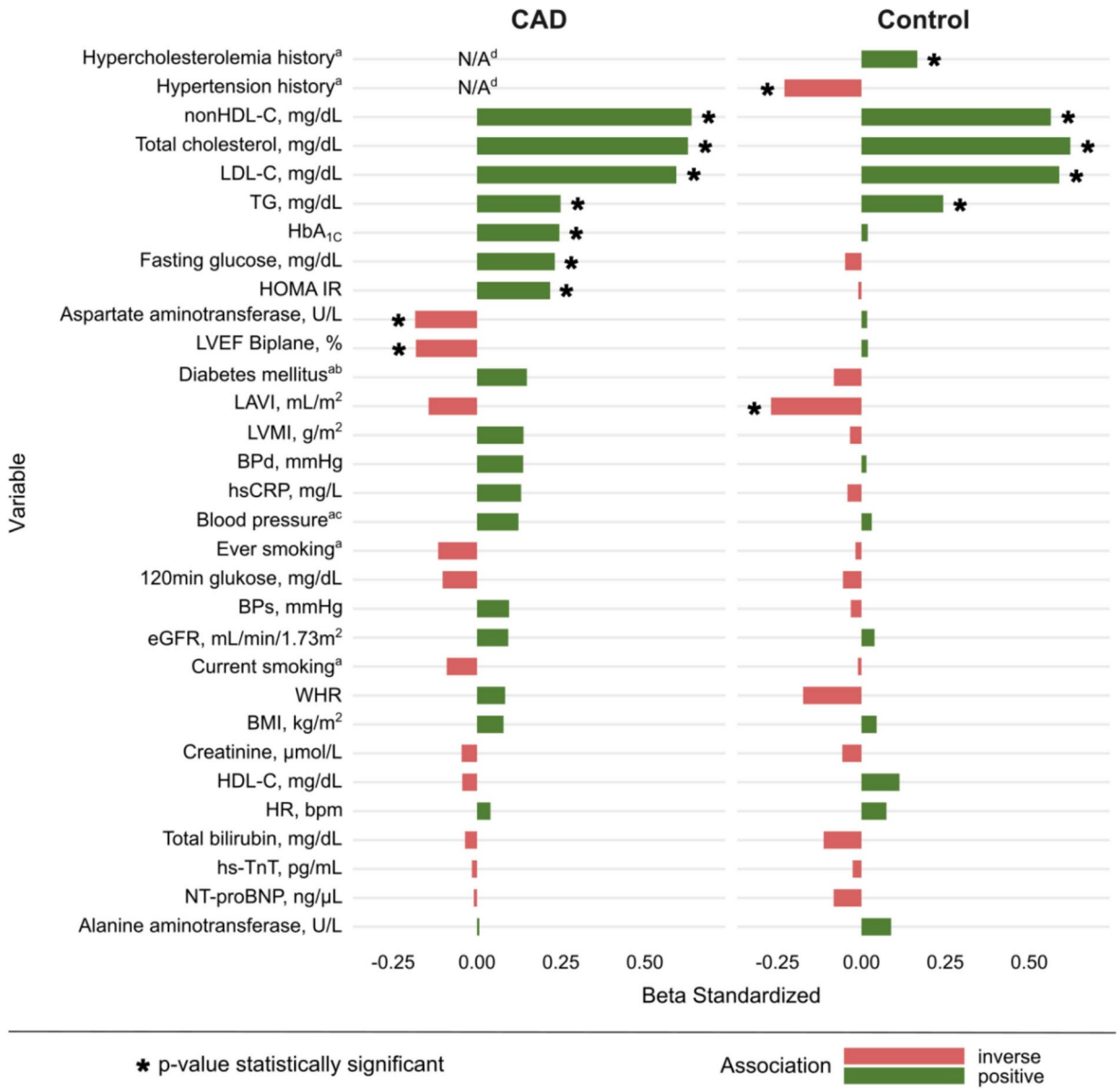

Fig. 3 Association of SM 41:1 concentration with clinical, echocardiographic, and laboratory parameters; [a]Categorical variable; [b]History or fasting glucose ≥ 126 mg/dL or OGTT120 min glucose ≥ 200 mg/dL or HbA1c ≥ 6.5%; [c]Systolic > 140 mmHg and/or diastolic > 90 mmHg; [d]The analysis was not performed since almost the entire group declared a disease; *BMI* body mass index, *BPd* blood pressure dystolic, *BPs* blood pressure systolic, *eGFR* estimated glomerular filtration rate, *HbA1c* glycated haemoglobin A1c, *HDL-C* high density lipoprotein cholesterol, *HR* heart rate, *hsCRP* high sensitivity C-reactive protein, *hs-TnT* high sensitivity troponin T., *LAVI* left atrial volume index, *LDL-C* low density lipoprotein cholesterol, *LVEF* left ventricular ejection fraction, *LVMI* left ventricular mass index, *nonHDL-C* non high density lipoprotein cholesterol, *NT-proBNP* n-terminal pro-brain natriuretic peptide, *TG* triglicerydes, *WHR* waist-hip ratio

4 Discussion

The plasma metabolome reflects the overall metabolic homeostasis resulting from the interactive effects of all metabolism-influencing genetic factors (Illig et al., 2010), environmental factors (Vernon et al., 2019), diet (Li et al., 2020), and health status (Newgard, 2017). Leveraging data identified a metabolite that significantly differs population with CAD from a healthy population, independently of known CVD risk factors. The ability to identify CAD independently of other CAD risk factors is a promising addition to the traditional stratification of individuals and potentially facilitates personalised medical interventions.

Sphingomyelin is a type of sphingolipid found in animal cell membranes, and its proper biosynthesis is essential for cell function. It usually consists of phosphorylcholine and ceramide (Maceyka & Spiegel, 2014). In humans, sphingomyelin is the only membrane phospholipid not derived from

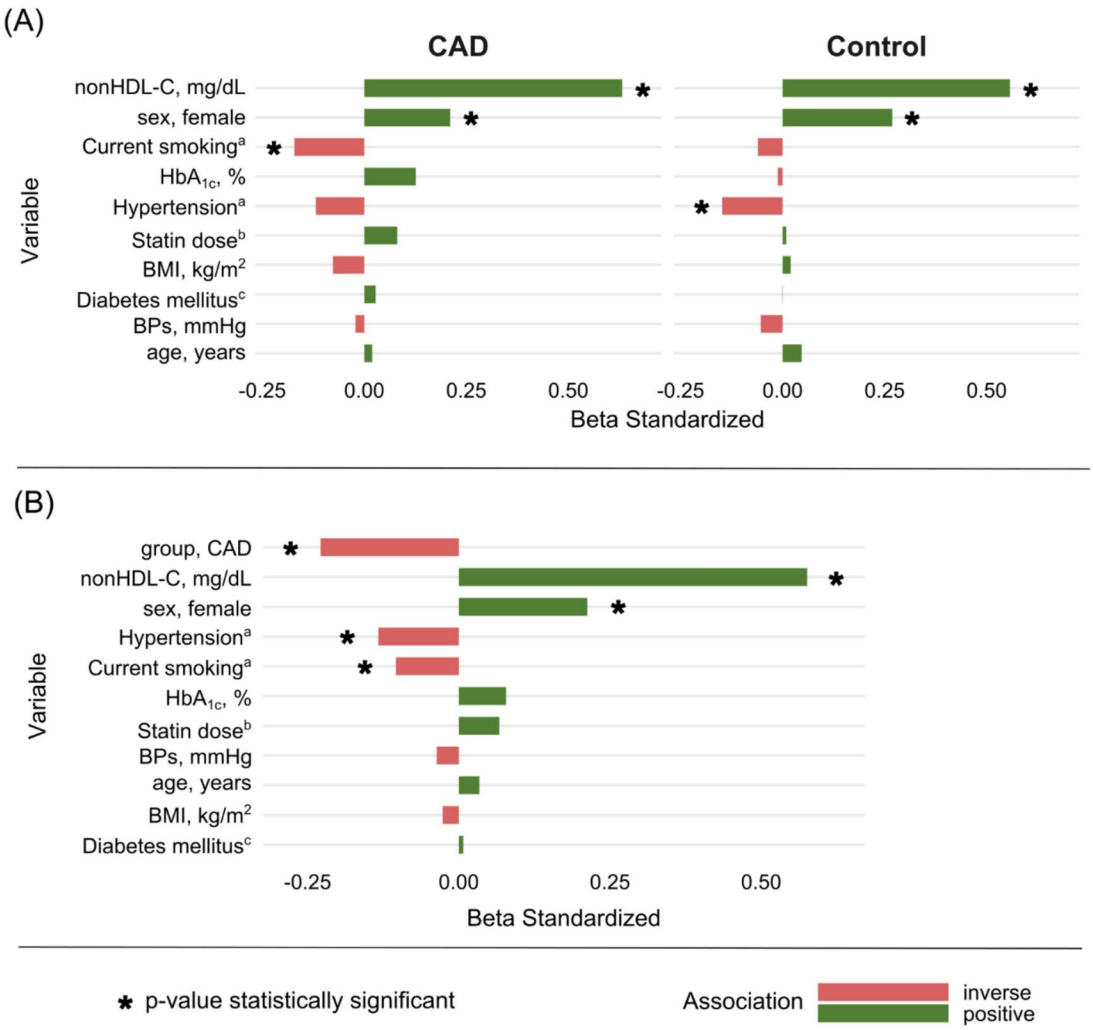

Fig. 4 Association of SM 41:1 concentration with study group, age, sex, body mass index (BMI), blood pressure systolic (BPs), hypertension, diabetes, smoking status, non high density lipoprotein cholesterol (nonHDL-C), glycated haemoglobin A1c (HbA1c), and statin dose equivalent to simvastatin. Panel **A** presents multiple regression model for sphingomyeline 41:1 (SM 41:1) (Model 2) separately performed for coronary artery disease (CAD) and control group. Panel **B** presents multiple regression model sphingomyeline 41:1 (SM 41:1) (Model 3) for entire study population. [a]Categorical variable; [b]Equivalent to simvastatin. [c]History or fasting glucose ≥ 126 mg/dL or OGGT 120 min glucose ≥ 200 mg/dL or HbA1c ≥ 6.5%

glycerol. Sphingomyelins are synthesised by the transfer of phosphorylcholine from phosphatidylcholine to ceramide in a reaction catalysed by sphingomyelin synthase (Maceyka & Spiegel, 2014). Sphingomyelins are components of cell membranes, playing crucial roles in maintaining structural integrity and facilitating cellular signalling. They are involved in various physiological processes, including cell growth, differentiation, and apoptosis regulation (Murai, 2025). Sphingomyelins also contribute to the formation of lipid rafts, which are essential for cellular communication and signalling pathways (Chakraborty & Jiang, 2013). Moreover, sphingomyelin is the main sphingolipid in HDL particles, and its amount in serum HDL influences reverse cholesterol transport (Zalloua et al., 2019). Sphingolipids have been shown to influence cellular activity, leading to insulin resistance, pancreatic disease, cardiomyopathy, and vascular disease. Inhibition of enzymatic sphingolipid synthesis has been shown to limit insulin resistance, diabetes, and atherosclerosis (Holland & Summers, 2008). In turn, increased sphingolipid synthesis promotes cell death by stressing the endoplasmic reticulum. This important organelle is responsible for lipid synthesis, and improper functioning can lead to insulin resistance, which in turn results in type 2 diabetes (Ross et al., 2014). Aslan et al. (2023) reported elevated sphingolipids, including sphingomyelins, in type 2 diabetic patients with LDL-C above 160 mg/dl, compared to patients with LDL-C below 100 mg/dl. Furthermore, Feng et al. (2024) identified lipids demonstrating the high diagnostic potential for type 2 diabetes. Sphingolipids, such as C16 sphingosine, C16 sphinganine,

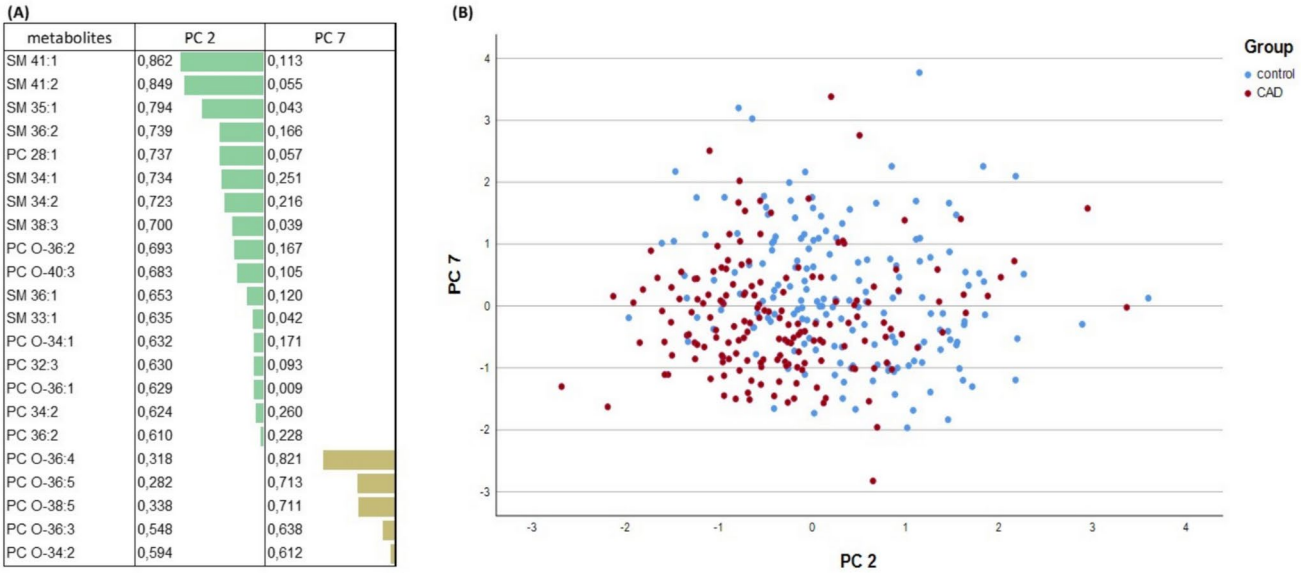

Fig. 5 PCA analysis: **A** Factor loadings of two principal components (PC) most differentiating coronary artery diseases (CAD) and healthy subjects, including metabolites with factor loading > 0.6, **B** PCA biplot of 2 factors differentiating CAD and healthy subjects the most

SM(d18:2/24:1) and SM(d18:1/24:1), were down-regulated, while C17 sphinganine and C17 Sphingosine were up-regulated relatively to controls [doi.org/https://doi.org/10.3390/metabo14110610]. Sphingolipids play a key role in cardiac function, both under physiological and pathological conditions (Borodzicz-Jazdzyk et al., 2022). Ceramide and sphingosine 1-phosphate (S1P) have modulated cardiomyocyte contractility in opposing ways. S1P exerts a negative inotropic effect, while ceramide enhances contractile function (Borodzicz-Jazdzyk et al., 2022). Lipid accumulation in the heart can cause impaired systolic function (Holland & Summers, 2008). Furthermore, it has been demonstrated that the accumulation of sphingolipids, such as ceramides and SM, in or around the heart can lead to dilated cardiomyopathy (Kovilakath & Cowart, 2020).

There are many reports in the literature on the connection between sphingomyelins and CAD, however, these results are not consistent. In our study, serum SM 41:1 concentration was inversely associated with CAD, although it was positively correlated with the level of non-HDL lipid fractions. Zalloua et al. (2019) report that sphingomyelin showed the strongest association with CAD incidence and the number of coronary stenoses. They also emphasised that sphingolipids alter LDL function in tissues, where LDL-associated sphingomyelins affect their LDL aggregation and accumulation in macrophages, as well as LDL kinetics. The reason for this is that sphingomyelinase in atherosclerotic plaques converts sphingomyelin into ceramide, which helps in the aggregation and uptake of LDL (Zalloua et al., 2019). Likewise, low HDL levels increase the probability of CAD (Sutter et al., 2015). HDL might lose cholesterol reverse transport properties and anti-inflammatory and anti-apoptotic properties. This is due to the transformation of HDL molecule induced by acute phase or chronic inflammation (Shao et al., 2012; Undurti et al., 2009), which in turn can be linked to the partial loss of sphingomyelins, in particular SM 33:1 and SM 38:1 as well as SM 42:1, from HDL molecules (Pruzanski et al., 2000). In the study conducted by Sutter et al., it was presented that levels of SM 42:1 associated with HDL are lower in patients with CAD and acute coronary syndrome than in healthy controls (Sutter et al., 2015). Djekic et al., presented an association between serum sphingomyelin intensity and calcium coronary artery disease. The intensities of the three sphingomyelins SM(d18:1/16:0), SM(d18:1/23:0), and SM(d18:1/16:0) were significantly lower in the group of subjects with severe calcification compared to those with no calcification. Similar results were obtained in two different laboratories participating in the experiment, demonstrating the clinical value of this study. Also noteworthy, the largest statistically significant difference was observed in the intensity of SM(d18:1/23:0) between patients with severely calcified coronary artery disease and those without calcification (Djekic et al., 2016).

SM has also been shown to be associated with other CAD factors, such as hypertension. Nannan Dai et al. (2023) demonstrated that systolic and diastolic blood pressure could decrease the tricosanoyl SM(d18:1/23:0) in the blood, suggesting potential therapeutic targets for hypertension intervention. Hannun showed (Hannun & Obeid, 2018), that decreased SMs may affect hypertension through regulating signal transduction and endothelial nitric oxide synthase via CERs. Our observed associations appeared to be consistent

with previous studies reporting that hypertensive individuals had deceased SM levels.

Some studies have shown, similarly to ours, higher SM concentrations in a healthy population. Nada Assi et al. (2018) showed that the lifestyle component's partial least squares score was negatively associated with lifetime alcohol, BMI, smoking, and diabetes and positively associated with physical activity. Its metabolic counterpart was positively related to the sphingomyelin metabolites, namely SM(OH)C14:1, SM(OH) C16:1, and SM(OH) C22:2. The sphingolipidome is extremely diverse and complex. Therefore, more studies are needed to determine how sphingomyelins are implicated in CVD.

In our study the metabolites concentrations also were explored by use of PCA method. It is a linear dimensionality reduction technique to reduce a large set of variables to a smaller set that still contains most of the original information. This method is already being used in medical science. Ricciardi et al. (2020) showed a practical implementation of traditional mining data techniques and PCA methods to predict CAD and to help clinicians in decision-making. We assessed how extracted factors differentiate the CAD and control groups. We showed that sphingomyelins and glycerophospholipids had the highest loadings of two PC most differentiating CAD and healthy subjects. In addition, SM 41.1 has the highest loadings in PC, which most strongly differentiates the study groups.

In conclusion, SM 41:1 is a sphingolipid species that has been implicated in the development and progression of CHD and atherosclerosis. Our study revealed significantly lower SM 41:1 concentration in the CAD group compared to individuals without cardiovascular events, regardless of known CV risk factors. In addition, using PCA analysis, we showed that the metabolome has the potential to differentiate between the CAD group and healthy subjects, of which SM 41.1 was the most important. These findings highlight the intricacies of the development of CAD. Consequently, measuring SM 41:1 level may help identify individuals at greater risk for atherosclerosis and related CVD complications, highlighting its potential utility as a biomarker and therapeutic target in CVD. Understanding the exact role of sphingolipid metabolites will enable the development of new risk stratifications or treatments.

4.1 Study limitations

A potential selection bias may exist in the study and control groups, as not all individuals who met the eligibility criteria ultimately participated in the study. The response rate in the study group was 73.6%, while in the Bialystok PLUS study it was 36.4%.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11306-025-02253-z.

Acknowledgements We thank Joanna Godzien, PhD for help with obtaining high resolution spectra of SM 41:1.

Author contributions M.K.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing original draft; M.Chlab.: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing original draft; J.J.: Formal analysis, Methodology, Software, Validation; N.Z.: Investigation; M.Cib.: Methodology, Supervision, Writing original draft, Writing review & editing; A.G.: Data curation, Formal analysis, Investigation, Methodology, Software, Writing original draft; E.S.Ś.: Investigation; K.P.: Investigation; M.Ł.: Investigation; A.K.: Project administration, Resources, Supervision, Writing review & editing; K.A.K.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing review & editing. All authors reviewed the manuscript.

Funding This work was supported by the Medical University of Bialystok, Poland [grant number SUB/1/00/19/001/1201] to [K.A.K].

Data availability The datasets are not publicly available because the individual privacy of the participants should be protected. However, data are available from the corresponding author upon reasonable request. The data supporting the findings of this study are available as part of the work and are included in the Supplementary Material 3.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval The study was approved by the Medical University of Bialystok (Poland) Bioethics Committee (IDs of the approvals: for study population R-I-002/323/2016 and for control group R-I-002/108/2016). The study was performed in accordance with the ethical standards put forward in the 1964 Declaration of Helsinki. Written informed consent for specimen collection was obtained from all participants.

References

Adamska-Patruno, E., Samczuk, P., Ciborowski, M., Godzien, J., Pietrowska, K., Bauer, W., Gorska, M., Barbas, C., & Kretowski,

A. (2019). Metabolomics reveal altered postprandial lipid metabolism after a high-carbohydrate meal in men at high genetic risk of diabetes. *Journal of Nutrition,149*, 915–922.

Aslan, I., Aydin, D., Koca, Y., Yilmaz, C., Ceker, T., Oztuzun, A., & Aslan, M. (2023). Sphingolipidomic profile and HDL subfractions in obese dyslipidemic type 2 diabetic patients. *Prostaglandins & Other Lipid Mediators,166*, 106719.

Assi, N., Gunter, M. J., Thomas, D. C., Leitzmann, M., Stepien, M., Chajes, V., Philip, T., Vineis, P., Bamia, C., Boutron-Ruault, M. C., Sandanger, T. M., Molinuevo, A., Boshuizen, H., Sundkvist, A., Kuhn, T., Travis, R., Overvad, K., Riboli, E., Scalbert, A.,… Ferrari, P. (2018). Metabolic signature of healthy lifestyle and its relation with risk of hepatocellular carcinoma in a large European cohort. *American Journal of Clinical Nutrition,108*, 117–126.

Borodzicz-Jazdzyk, S., Jazdzyk, P., Lysik, W., Cudnoch-Jedrzejewska, A., & Czarzasta, K. (2022). Sphingolipid metabolism and signaling in cardiovascular diseases. *Frontiers in Cardiovascular Medicine,9*, 915961.

Chakraborty, M., & Jiang, X. C. (2013). Sphingomyelin and its role in cellular signaling. *Advances in Experimental Medicine and Biology,991*, 1–14.

Chlabicz, M., Jamiolkowski, J., Laguna, W., Dubatowka, M., Sowa, P., Lapinska, M., Szpakowicz, A., Zieleniewska, N., Zalewska, M., Raczkowski, A., & Kaminski, K. A. (2022). Effectiveness of lifestyle modification vs. therapeutic, preventative strategies for reducing cardiovascular risk in primary prevention-A cohort study. *Journal of Clinical Medicine,11*, 688.

Chlabicz, M., Jamiołkowski, J., Sowa, P., Zalewska, M., Kiszkiel, Ł, Ciołkiewicz, M., Motkowski, R., Kowalska, I., Minarowski, Ł, & Kamiński, K. A. (2023). Multimorbidity patterns in the urban population in Poland. *Journal of Clinical Medicine,12*(18), 5860.

Dai, N., Deng, Y., & Wang, B. (2023). Association between human blood metabolome and the risk of hypertension. *BMC Genomic Data,24*, 79.

Dawber, T. R., Moore, F. E., & Mann, G. V. (1957). Coronary heart disease in the Framingham study. *American Journal of Public Health and the Nation's Health,47*, 4–24.

Djekic, D., Pinto, R., Vorkas, P. A., & Henein, M. Y. (2016). Replication of LC-MS untargeted lipidomics results in patients with calcific coronary disease: An interlaboratory reproducibility study. *International Journal of Cardiology,222*, 1042–1048.

Feng, L., He, B., Xia, J., & Wang, Z. (2024). Untargeted and targeted lipidomics unveil dynamic lipid metabolism alterations in type 2 diabetes. *Metabolites,14*(11), 610.

Godlewski, A., Czajkowski, M., Mojsak, P., Pienkowski, T., Gosk, W., Lyson, T., Mariak, Z., Reszec, J., Kondraciuk, M., Kaminski, K., Kretowski, M., Moniuszko, M., Kretowski, A., & Ciborowski, M. (2023). A comparison of different machine-learning techniques for the selection of a panel of metabolites allowing early detection of brain tumors. *Science and Reports,13*, 11044.

Hannun, Y. A., & Obeid, L. M. (2018). Sphingolipids and their metabolism in physiology and disease. *Nature Reviews Molecular Cell Biology,19*, 175–191.

Holland, W. L., & Summers, S. A. (2008). Sphingolipids, insulin resistance, and metabolic disease: New insights from in vivo manipulation of sphingolipid metabolism. *Endocrine Reviews,29*, 381–402.

Illig, T., Gieger, C., Zhai, G., Romisch-Margl, W., Wang-Sattler, R., Prehn, C., Altmaier, E., Kastenmuller, G., Kato, B. S., Mewes, H. W., Meitinger, T., de Angelis, M. H., Kronenberg, F., Soranzo, N., Wichmann, H. E., Spector, T. D., Adamski, J., & Suhre, K. (2010). A genome-wide perspective of genetic variation in human metabolism. *Nature Genetics,42*, 137–141.

Johnson, C. H., Ivanisevic, J., & Siuzdak, G. (2016). Metabolomics: Beyond biomarkers and towards mechanisms. *Nature Reviews Molecular Cell Biology,17*, 451–459.

Kovilakath, A., & Cowart, L. A. (2020). Sphingolipid mediators of myocardial pathology. *Journal of Lipid and Atherosclerosis,9*, 23–49.

Li, J., Guasch-Ferre, M., Chung, W., Ruiz-Canela, M., Toledo, E., Corella, D., Bhupathiraju, S. N., Tobias, D. K., Tabung, F. K., Hu, J., Zhao, T., Turman, C., Feng, Y. A., Clish, C. B., Mucci, L., Eliassen, A. H., Costenbader, K. H., Karlson, E. W., Wolpin, B. M.,… Liang, L. (2020). The Mediterranean diet, plasma metabolome, and cardiovascular disease risk. *European Heart Journal,41*, 2645–2656.

Lv, J., Pan, C., Cai, Y., Han, X., Wang, C., Ma, J., Pang, J., Xu, F., Wu, S., Kou, T., Ren, F., Zhu, Z. J., Zhang, T., Wang, J., & Chen, Y. (2024). Plasma metabolomics reveals the shared and distinct metabolic disturbances associated with cardiovascular events in coronary artery disease. *Nature Communications,15*, 5729.

Maceyka, M., & Spiegel, S. (2014). Sphingolipid metabolites in inflammatory disease. *Nature,510*, 58–67.

Mahmood, S. S., Levy, D., Vasan, R. S., & Wang, T. J. (2014). The Framingham heart study and the epidemiology of cardiovascular disease: A historical perspective. *Lancet,383*, 999–1008.

Marcinkiewicz-Siemion, M., Ciborowski, M., Ptaszynska-Kopczynska, K., Szpakowicz, A., Lisowska, A., Jasiewicz, M., Waszkiewicz, E., Kretowski, A., Musial, W. J., & Kaminski, K. A. (2018). LC-MS-based serum fingerprinting reveals significant dysregulation of phospholipids in chronic heart failure. *Journal of Pharmaceutical and Biomedical Analysis,154*, 354–363.

Murai, Y. (2025). Elucidation of physiological functions of sphingolipid-related molecules by chemical approaches. *Bioscience, Biotechnology, and Biochemistry,89*, 205–214.

Newgard, C. B. (2017). Metabolomics and metabolic diseases: Where do we stand? *Cell Metabolism,25*, 43–56.

Pikala, M., & Maniecka-Bryla, I. (2017). Fifteen-year mortality trends due to cardiovascular diseases in Poland using standard expected years of life lost, 2000–2014. *Kardiologia Polska,75*, 1033–1040.

Pinaire, J., Aze, J., Bringay, S., Cayla, G., & Landais, P. (2019). Hospital burden of coronary artery disease: Trends of myocardial infarction and/or percutaneous coronary interventions in France 2009–2014. *PLoS ONE,14*, e0215649.

Pruzanski, W., Stefanski, E., de Beer, F. C., de Beer, M. C., Ravandi, A., & Kuksis, A. (2000). Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. *Journal of Lipid Research,41*, 1035–1047.

Ricciardi, C., Valente, A. S., Edmund, K., Cantoni, V., Green, R., Fiorillo, A., Picone, I., Santini, S., & Cesarelli, M. (2020). Linear discriminant analysis and principal component analysis to predict coronary artery disease. *Health Informatics Journal,26*, 2181–2192.

Ross, J. S., Russo, S. B., Chavis, G. C., & Cowart, L. A. (2014). Sphingolipid regulators of cellular dysfunction in Type 2 diabetes mellitus: A systems overview. *Clinical Lipidology,9*, 553–569.

Sawicka-Smiarowska, E., Bondarczuk, K., Bauer, W., Niemira, M., Szalkowska, A., Raczkowska, J., Kwasniewski, M., Tarasiuk, E., Dubatowka, M., Lapinska, M., Szpakowicz, M., Stachurska, Z., Szpakowicz, A., Sowa, P., Raczkowski, A., Kondraciuk, M., Gierej, M., Motyka, J., Jamiolkowski, J.,… Kaminski, K. A. (2021). Gut microbiome in chronic coronary syndrome patients. *Journal of Clinical Medicine,10*(21), 5074.

Shao, B., Pennathur, S., & Heinecke, J. W. (2012). Myeloperoxidase targets apolipoprotein A-I, the major high density lipoprotein protein, for site-specific oxidation in human atherosclerotic lesions. *Journal of Biological Chemistry,287*, 6375–6386.

Sutter, I., Velagapudi, S., Othman, A., Riwanto, M., Manz, J., Rohrer, L., Rentsch, K., Hornemann, T., Landmesser, U., & von Eckardstein, A. (2015). Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL. *Atherosclerosis,241*, 539–546.

Undurti, A., Huang, Y., Lupica, J. A., Smith, J. D., DiDonato, J. A., & Hazen, S. L. (2009). Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. *Journal of Biological Chemistry,284*, 30825–30835.

Vernon, S. T., Hansen, T., Kott, K. A., Yang, J. Y., O'Sullivan, J. F., & Figtree, G. A. (2019). Utilizing state-of-the-art "omics" technology and bioinformatics to identify new biological mechanisms and biomarkers for coronary artery disease. *Microcirculation,26*, e12488.

Vernon, S. T., Tang, O., Kim, T., Chan, A. S., Kott, K. A., Park, J., Hansen, T., Koay, Y. C., Grieve, S. M., O'Sullivan, J. F., Yang, J. Y., & Figtree, G. A. (2021). Metabolic signatures in coronary artery disease: Results from the BioHEART-CT study. *Cells,10*, 980.

Wang, T. J. (2011). Assessing the role of circulating, genetic, and imaging biomarkers in cardiovascular risk prediction. *Circulation,123*, 551–565.

Wilkins, E., Wilson, L., Wickramasinghe, K., Bhatnagar, P., Leal, J., Luengo-Fernandez, R., Burns, R., Rayner, M., Townsend, N (2017) European cardiovascular disease statistics 2017 in Network, E.H. (Ed).

Zalloua, P., Kadar, H., Hariri, E., Abi Farraj, L., Brial, F., Hedjazi, L., Le Lay, A., Colleu, A., Dubus, J., Touboul, D., Matsuda, F., Lathrop, M., Nicholson, J. K., Dumas, M. E., & Gauguier, D. (2019). Untargeted mass spectrometry lipidomics identifies correlation between serum sphingomyelins and plasma cholesterol. *Lipids in Health and Disease,18*, 38.