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ORIGINAL ARTICLE

Metabolomics signature associated with circulating serum selenoprotein P levels

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Abstract

Purpose Selenoprotein P (SELENOP) has been previously related to various metabolic traits with partially conflicting results. The identification of SELENOP-associated metabolites, using an untargeted metabolomics approach, may provide novel biological insights relevant to disentangle the role of SELENOP in human health.

Methods In this cross-sectional study, 572 serum metabolites were identified by comparing the obtained LC–MS/MS spectra with spectra stored in Metabolon's spectra library. Serum SELENOP levels were measured in 832 men and women using an ELISA kit.

Results Circulating SELENOP levels were associated with 24 out of 572 metabolites after accounting for the number of independent dimensions in the metabolomics data, including inverse associations with alanine, glutamate, leucine, isoleucine and valine, an unknown compound X-12063, urate and the peptides gamma-glutamyl-leucine, and N-acetylcarnosine. Positive associations were observed between SELENOP and several lipid compounds. Of the identified metabolites, each standard deviation increase in the branched-chain amino acids (isoleucine, leucine, valine), alanine and gamma-glutamyl-leucine was related to higher odds of having T2DM [OR (95% CI): 1.96 (1.41–2.73); 1.62 (1.15–2.28); 1.94 (1.45–2.60), 1.57 (1.17–2.11), and 1.52 (1.13–2.05), respectively].

Conclusions Higher serum SELENOP levels were associated with an overall healthy metabolomics profile, which may provide further insights into potential mechanisms of SELENOP-associated metabolic disorders.

Keywords Hepatokine · SELENOP · Untargeted metabolomics · Metabolic disorders · PopGen

Introduction

Selenoprotein P (SELENOP), the central protein for Se metabolism and transport from the liver to extrahepatic tissues [1], has been suggested to play a role in glucose metabolism and insulin sensitivity. Results from cross-sectional studies have reported elevated SELENOP levels in patients with type 2 diabetes (T2DM) [2, 3], and observed moderate positive correlations between SELENOP levels and fasting plasma glucose among T2DM patients [4], in contrast to more recent epidemiologic findings showing inverse relations of SELENOP levels with several metabolic traits in adults [5, 6] and young children [7]. Moreover,

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circulating SELENOP levels were positively related to several cardiometabolic traits and biomarkers, including C-reactive protein, brachial-ankle pulse wave velocity, and with visceral fat area [8].

However, whether SELENOP actually confers independent clinical prognostic value for T2DM, and other metabolic-related disorders, or whether it is merely a bystander in this association is difficult to disentangle from these controversial studies.

For instance, the higher SELENOP levels observed in patients with T2DM, may be secondary to an increased Se transport demand due to the Se deficiency occurring with this condition [9, 10]. On the other hand, circulating SELENOP as a negative acute phase reactant is down-regulated by inflammation [11]. Therefore, the lower levels observed, for example, in individuals with the metabolic syndrome (MetS) might be explained by their higher inflammatory state.

Yet, SELENOP has two essential properties to maintain Se-homeostasis and to protect extracellular compartments against oxidative damage during inflammation [1]. For instance, in a cell-free in-vitro system SELENOP was shown to protect low-density lipoprotein against oxidation [12], and to protect endothelial cells from oxidative damage by stimulating the expression and activity of glutathione peroxidase [13]. Furthermore, because of its putative anti-oxidant function, SELENOP is thought to play a role in cancer prevention [14]. However, so far, the antioxidant properties of SELENOP have not been fully explored and characterized [15], and this calls for a more in-depth understanding of the chemical roles and biological mechanisms of this protein.

Metabolomics, a technology used to identify small molecule metabolites in a biospecimen, is emerging for its features to identify and characterize metabolites associated with biological pathways and, thereby, unraveling potential mechanisms influencing human health.

Therefore, with the intent to characterize biological pathways related to SELENOP, we aimed to identify metabolites, by means of an untargeted approach, that are linked to circulating SELENOP levels. Furthermore, we aimed to relate the identified metabolomics profile to two major metabolic disorders previously linked to SELENOP metabolism, i.e., prevalent T2DM and the MetS.

Subjects and methods

Study design and population

Between 2005 and 2007, 1316 individuals of the general population in Kiel, Northern Germany, were recruited by the PopGen biobank [16]. At the second examination cycle (2010–2012), 952 individuals received comprehensive clinical and molecular phenotyping along with blood sampling. All participants filled-in medical and lifestyle questionnaires, as described elsewhere [16, 17].

For the present cross-sectional analysis, we used the data from the first follow-up examination. Serum metabolites were measured from 855 participants, and after exclusion of participants with missing SELENOP levels ($n = 2$) and other covariates ($n = 21$), the final study sample consisted of 832 participants.

The study procedures have been approved by the Ethics Committee of the Medical Faculty of the University of Kiel, Germany. All participants provided written informed consent prior to their inclusion in the study.

Biochemical measurements

Metabolomics profiling

Fasting blood samples were obtained from participants in a sitting position. Blood samples for biomarker analyses in serum were drawn into serum separator tubes (Sarstedt AG, Germany), centrifuged, aliquoted, and stored at −80 °C until analyses.

Serum metabolites of 855 participants were measured in two different sample sets, at the Helmholtz Zentrum München, using a untargeted approach, as described elsewhere [18]. The laboratory staff was blinded regarding the clinical information corresponding to the study samples.

Briefly, 100 µL of the serum were pipetted into a 2 mL 96-well plate. 475 µL of methanol were used to extract serum samples metabolites. After centrifugation, four aliquots of the supernatant, each 100 µL, were split onto two 96-well microplates. The first two aliquots were used for LC–MS/MS analysis in positive and negative electrospray ionization, whereas the others were kept as reserves. Besides the participants' samples, human reference plasma and a pooled of human serum samples were extracted in 1 and 6 wells of the 96-well plate, respectively. These samples served as technical replicates throughout the data set to assess process variability. Based on 116 reference serum aliquots the mean coefficient of variation for the measured metabolites was 15.8%. LC–MS/MS analysis was performed on a linear ion trap LTQ XL mass spectrometer (Thermo Fisher Scientific, Germany) coupled with a Waters Acquity UPLC system (Waters, Germany). The metabolites were identified by comparing the obtained LC–MS/MS spectra with spectra stored in Metabolon's spectra library (Metabolon, USA) [19].

A total of 645 metabolites were identified and categorized into amino acid, carbohydrate, cofactors and vitamins, energy metabolites, lipids, nucleotides, peptides, xenobiotics, and unknown compounds.

Measurement of SELENOP in serum

Serum SELENOP concentrations were measured between July and December 2014 with a quantitative sandwich enzyme immunoassay (Cusabio CSB-EL021018HU), with intra-assay and inter-assay coefficient of variations of <8% and <10%, respectively. To assess the validity of SELENOP levels, in January 2018, we re-measured circulating SELENOP levels in a subset of 20 participants with another assay (Abbexa, abx251264, Human Selenoprotein P1, ELISA Kit). Those 20 samples were run in duplicate and averaged, with a duplicate intra-assay CV of 4.9%. We observed a good positive correlation between the two ELISA measurements (Spearman correlation coefficient = 0.73), suggesting valid SELENOP measurements.

Routine laboratory measurements

EDTA plasma glucose, glycated hemoglobin (HbA1c), total-cholesterol, HDL-cholesterol, and LDL-cholesterol, triglycerides, and lithium heparin plasma C-reactive protein (CRP) were measured on the same day in unfrozen blood samples at the Institute for Clinical Chemistry, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany [20].

Examination protocol and definitions

Trained personnel performed anthropometric measurements [17, 21]. Body weight and height were measured with participants wearing light indoor clothing and no shoes. BMI was calculated as kg/m^2, and waist circumferences were measured at the midpoint between the lower costal margin and the superior iliac crest [21].

With the participants sitting for at least 5 min, blood pressure measures were taken twice with a sphygmomanometer, and then averaged. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or use of antihypertensive medication. Type 2 diabetes was defined based on medication records, or HbA1c ≥ 6.5% (48 mmol/mol), or fasting serum glucose ≥ 126 mg/dL.

Physical activity was assessed using a validated questionnaire and classified in metabolic equivalent (MET)-h/week [21, 22].

The metabolic syndrome (MetS) was defined according to the harmonized ATP III criteria [23] based on the presence of at least three of the following criteria: (1) abdominal obesity (waist circumference ≥ 94 cm for men or ≥80 cm for women), (2) elevated triglycerides ≥ 150 mg/dL, (3) reduced HDL-cholesterol ≤ 40 mg/dL for men or 50 mg/dL for women, (4) elevated systolic/diastolic blood pressure ≥ 130/85 mmHg or receiving drug treatment, and (5) elevated fasting plasma glucose ≥ 100 mg/dL or antidiabetic medications [23]. Since information on triglyceride-lowering and HDL-raising medications were not available, these data were not included in our definition.

Statistical analysis

The raw ion counts for the 645 identified metabolites were divided by the median value of the samples' run day (34 samples per run day) to correct for day-to-day variance.

Upon normalization, missing metabolite values were imputed with the minimum detected value. For the main analyses, all metabolites were natural log-transformed due to their right skewed distribution. However, metabolites with more than 80% missing values ($n = 73$) were excluded thus leaving 572 metabolites available for the analyses.

To assess whether a batch effect was present, we first visually inspected the plot of the first two principal components and we calculated the average Bhattacharyya distance for the sample set of the two batches [24]; the smaller the average, the smaller the batch effects. In the present paper, the average Bhattacharyya distance was -5.92×10^{-17}, thus confirming the absence of a batch effect as also observed from the correlation plot of the two principal components (data not shown).

Nonparametric correlations between 572 metabolites and serum SELENOP levels were examined using Spearman's correlation coefficients (Rho). Multivariable Tobit regression and linear regression models (respectively, for censored and fully observed metabolites levels) were used to assess the associations between each log-transformed metabolite (outcome) and continuous SELENOP levels (predictor). Tobit, i.e., censored regression, rather than least-squares regression was applied to avoid imputation. For Tobit regression, we used as left-censoring the smallest detected metabolite value. In the main analysis, the models were adjusted for age, sex, the residual of waist circumference regressed on BMI, physical activity, education level, smoking status, prevalent diabetes, and hypertension. The final models were furthermore forced by an additional adjustment for batch number.

To account for multiple testing, we used the number of principal components calculated using principal components analysis as proxy for the number of independent tests. We identified 135 components with eigenvalues higher than 1, explaining 80% of the variance in the metabolomics measures [25]. Therefore, the adjusted *P*-value threshold was set at 3.70×10^{-4} (0.05/135).

Stratified analyses by sex were additionally performed to assess possible differences in the associations of SELENOP with the metabolites between men and women.

Multivariable-adjusted logistic regression models were used to investigate the relations of the identified metabolites

Table 1 General characteristics of the study population

Characteristics[a]	Overall	Women	Men
n	832	358	474
Selenoprotein P, μg/mL	5.59 (3.87–8.32)	7.56 (5.29–11.1)	4.67 (3.32–6.23)
Age, yrs	60.6 (59.8–61.5)	60.4 (59.1–61.7)	60.8 (59.6–61.9)
Body mass index (kg/m^2)	27.3 (27.0–27.6)	26.8 (26.4–27.3)	27.6 (27.2–28.1)
Waist circumference, cm	96.4 (95.5–97.3)	90.6 (89.3–91.8)	100.8 (99.7–101.9)
Prevalent hypertension, %	62.4	57.3	66.2
Prevalent diabetes, %	9.4	5.6	12.2
Current smokers, %	13.0	12.5	13.4
Physical activity, MET-h/week	101.6 (97.4–105.8)	107.6 (101.2–114.0)	97.1 (91.5–102.6)
High education, %	32.6	25.4	38.0
Alcohol consumption, g/day	8.9 (3.2–18.5)	5.4 (2.0–12.2)	12.4 (5.6–23.4)

[a]Unadjusted median and interquartile range (IQR) (selenoprotein P and alcohol consumption), means and 95% confidence interval, and percentages (%)

(predictors) associated with SELENOP levels with binary outcomes (i.e., MetS and prevalent T2DM), and results were expressed as odds ratios (OR) and 95% CI. The models were adjusted for age, sex, physical activity, education level, smoking status, alcohol consumption, and mutually adjusted for the other metabolites. Prevalent diabetes was additionally adjusted for the residual of waist circumference regressed on BMI, hypertension, triglycerides, total-cholesterol, and HDL-cholesterol.

All analyses were performed with SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA).

Results

General characteristics of the 832 PopGen participants are shown in Table 1. Women had on average higher SELENOP levels (median 7.56 μg/mL, interquartile range (IQR): 5.29–11.1) than men (median 4.67 μg/mL, IQR: (3.32–6.23)), and the overall study sample was characterized by middle aged participants, who were on average overweight, with an history of hypertension. Men had a higher prevalence of T2DM, smoked more, exercised less than women, and had a higher educational level. On average both men and women consumed alcohol in moderation.

Spearman's correlation coefficients of each metabolite with SELENOP levels are reported in supplementary Table 1.

In the multivariable analysis, circulating SELENOP levels were associated with 142 out of 572 metabolites at nominal level ($p < 0.05$) and 24 out of 142 after accounting for multiple testing ($p < 0.000373$).

Modest intercorrelations were observed between these 24 metabolites (Fig. 1), with the highest correlation observed, as expected, among metabolites belonging to the same superpathway.

Overall, higher circulating SELENOP levels were associated with a metabolite profile characterized by inverse associations with alanine and glutamate as well as the branch chain amino acids (BCAAs) leucine, isoleucine, and valine (Table 2). An unknown compound X-12063, previously associated with insulin resistance (IR), showed the strongest inverse association at the PCA-threshold. Urate, a nucleotide, and the peptide gamma-glutamyl-leucine, and N-acetylcarnosine were also inversely related to circulating SELENOP levels (Table 2).

Positive associations were observed between SELENOP and several lipid compounds. Each 1-unit increment in SELENOP levels was associated with 0.996%, 0.941%, and 1.022% higher docosadienoic acid levels, nonadecanoate, and arachidate levels, respectively. Amongst the lysolipid subclass the 2-oleoyl-GPC, 2-linoleoyl-GPC, 1-dihomo-linoleoyl-GPC, and 1-linolenoyl-GPC also showed moderate to strong positive associations with SELENOP levels at the PCA-threshold. Each 1-unit increment in SELENOP levels was associated with 2.6%, 2.2%, 2.7%, and 1.5% higher dodecanedioate, decanedioate, hexadecanedioate, and octadecanedioate, respectively. Furthermore, positive associations were observed between SELENOP levels and three unknown compounds. In particular, each unit increment in SELENOP levels was associated with 1.8%, 3.6%, and 2.2% higher X-11538, X-16137, and X-17145 levels, respectively.

All PCA-threshold SELENOP-metabolite associations did not differ between men and women (data not shown).

Results of the multivariable-adjusted logistic regression analysis for prevalent diabetes and the MetS and all SELENOP-associated metabolites at the PCA-threshold are shown in Table 3. Of the identified metabolites, the BCAAs, alanine, and gamma-glutamyl-leucine showed strong positive associations with the prevalence of T2DM. In particular, each standard deviation increase in isoleucine, leucine, valine, alanine, and gamma-glutamyl-leucine was

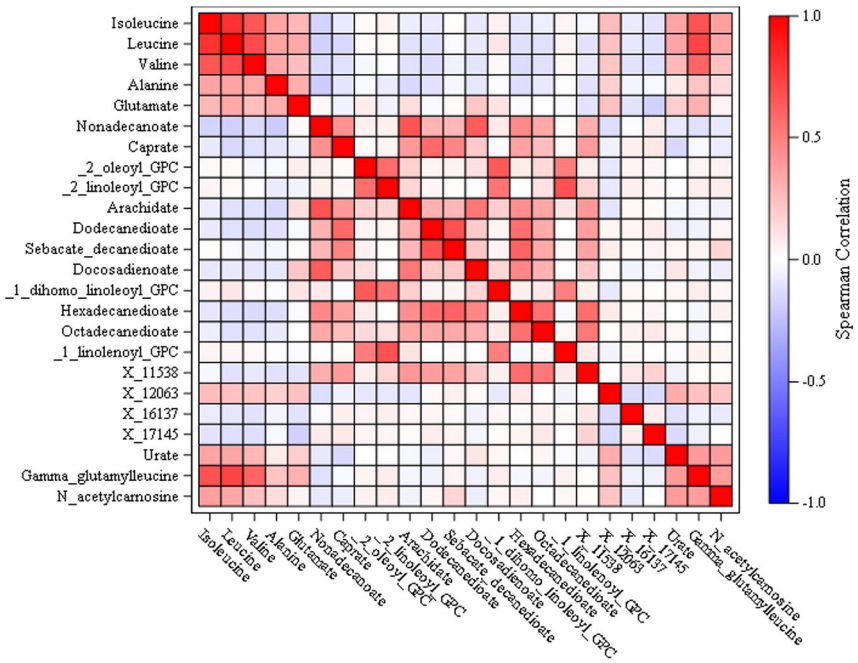

Fig. 1 Heat map of Spearman's correlations of selected metabolites associated with circulating SELENOP levels

associated with higher probabilities of having T2DM (OR: 1.96, 95% CI: 1.41-2.73; OR: 1.62, 95% CI: 1.15-2.28; OR: 1.94, 95% CI: 1.45-2.60; OR: 1.57, 95% CI: 1.17-2.11; OR: 1.52, 95% CI: 1.13-2.05, respectively). Among the identified unknown compounds positively related to SELENOP levels, X-11538 was associated with a higher probability of having T2DM (OR: 1.41, 95% CI: 1.00-1.99). Regarding the MetS, the nonadecanoate (19:0) (OR: 0.72, 95% CI: 0.53–0.97) and the 2-linoleoyl-GPC (18:2) (OR: 0.68, 95% CI: 0.51–0.90) showed inverse associations whereas isoleucine, docosadienoate (22:2n6), and gamma-glutamyl-leucine showed positive associations (respectively, OR: 1.28, 95% CI: 1.04-1.60; OR: 1.33, 95% CI: 1.01-1.75; OR: 1.31, 95% CI: 1.05-1.64). In view of the positive correlation observed between SELENOP and HDL-cholesterol levels (age and sex adjusted spearman rho = 0.41), also reported in a prior publication [5], and considering the observed associations of some lipids with the MetS, we further assessed potential relations of the lipids related to SELENOP with HDL-cholesterol levels only, since SELENOP was not associated with the LDL-c fraction (rho = 0.001). However, after multivariable-adjusted logistic regression analysis none of the identified lipids were associated with HDL-c (data not shown).

Discussion

Main observations

In the present analyses, using an untargeted approach with 572 candidate metabolomic markers, we identified 24 metabolites that were associated with circulating SELENOP concentrations in multivariable-adjusted statistical models and after correction for multiple testing.

Specifically, the metabolites inversely related to SELENOP levels included branched-chain and other amino acids, an unknown compound previously related to IR, as well as urate, gamma-glutamyl-leucine, and N-acetylcarnosine. Various lipids and three other unknwon metabolites were positively related to SELENOP levels, and the observed associations were generally similar in men and women. Of the identified metabolites, the BCAAs as well as alanine and gamma-glutamyl-leucine showed positive associations with T2DM prevalence in our sample, whereas nonadecanoate and 2-linoleoyl-GPC showed inverse associations with the MetS as opposed to the positive association observed for docosadienoate.

To the best of our knowledge, this is the first study to characterize a metabolomics signature associated with circulating serum SELENOP levels.

Associations of metabolomic markers with SELENOP concentrations

BCAAs and glutamate were inversely related to SELENOP. Prior studies have shown that these amino acids may contribute to the development of obesity-associated IR [26] and a pro-inflammatory state [27], both risk factors for T2DM and cardiovascular disease [28]. In line with these previous findings, we observed that among the identified metabolites the BCAAs and alanine showed strong positive associations with the prevalence of T2DM. Data from the Framingham Heart Study and the Malmö Study also indicate that BCAAs

Table 2 Beta coefficients and 95% confidence intervals (CI) of serum metabolites associated with SELENOP at principal component *p* value-corrected threshold

			SELENOP			
Metabolite	Superpathway	Subpathway	*β*-estimate[a]	LCL	UCL	*p*
Isoleucine	Amino acid	Leucine, isoleucine and valine metabolism	−0.0039	−0.0060	−0.0019	2.14E−04
Leucine	Amino acid	Leucine, isoleucine and valine metabolism	−0.0038	−0.0057	−0.0018	1.43E−04
Valine	Amino acid	Leucine, isoleucine and valine metabolism	−0.0045	−0.0065	−0.0024	2.02E−05
Alanine	Amino acid	Alanine and aspartate metabolism	−0.0064	−0.0091	−0.0037	2.80E−06
Glutamate	Amino acid	Glutamate metabolism	−0.0062	−0.0092	−0.0031	8.73E−05
Caprate (10:0)	Lipid	Medium chain fatty acid	0.0148	0.0077	0.0218	4.23E−05
Nonadecanoate (19:0)	Lipid	Long chain fatty acid	0.0094	0.0054	0.0133	3.24E−06
Arachidate (20:0)	Lipid	Long chain fatty acid	0.0102	0.0066	0.0139	4.98E−08
2-oleoyl-GPC (18:1)	Lipid	Lysolipid	0.0104	0.0058	0.0149	9.88E−06
2-linoleoyl-GPC (18:2)	Lipid	Lysolipid	0.0112	0.0058	0.0166	5.51E−05
1-linolenoyl-GPC (18:3)	Lipid	Lysolipid	0.0132	0.0061	0.0203	2.63E−04
1-dihomo-linoleoyl-GPC (20:2)	Lipid	Lysolipid	0.0103	0.0051	0.0155	9.84E−05
Dodecanedioate	Lipid	Fatty acid, dicarboxylate	0.0265	0.0127	0.0402	1.63E−04
Sebacate (decanedioate)	Lipid	Fatty acid, dicarboxylate	0.0225	0.0103	0.0346	2.83E−04
Hexadecanedioate	Lipid	Fatty acid, dicarboxylate	0.0272	0.0166	0.0379	5.63E−07
Octadecanedioate	Lipid	Fatty acid, dicarboxylate	0.0150	0.0080	0.0220	2.87E−05
Docosadienoate (22:2n6)	Lipid	Polyunsaturated fatty acid (n3 and n6)	0.0100	0.0046	0.0154	3.04E−04
X-11538	Unknown	Unknown	0.0183	0.0109	0.0257	1.24E−06
X-12063	Unknown	Unknown	−0.0306	−0.0415	−0.0197	2.94E−08
X-16137	Unknown	Unknown	0.03634	0.0173	0.0554	1.87E−04
X-17145	Unknown	Unknown	0.02162	0.0010	0.0335	3.55E−04
Urate	Nucleotide	Purine metabolism	−0.0040	−0.0061	−0.0019	2.19E−04
Gamma-glutamyl-leucine	Peptide	Gamma-glutamyl amino acid	−0.0046	−0.0071	−0.0021	3.70E−04
N-acetylcarnosine	Peptide	Dipeptide derivative	−0.0280	−0.0396	−0.0163	2.56E−06

[a]Adjusted for age, sex, the residual of waist circumference regressed on body mass index, physical activity, education level, smoking status, prevalent diabetes and hypertension, batch number, and mutually adjusted for the other metabolites

and glutamate were positively associated with several metabolic risk factors, including IR, high blood pressure, dyslipidemia, and obesity [29]. A possible explanation of the inverse associations observed in the present study between circulating SELENOP levels and these metabolites could be explained by a compensatory higher SELENOP expression in response to a reduced inflammatory burden and oxidative stress, as a consequence of the lower circulating levels of these metabolites. In particular, since the first selenocystein residue towards the N-terminus of SELENOP has been suggested to confer antioxidant capacity to cells, we may formulate that local sources of SELENOP could play a role in mitigating oxidative stress during metabolic disorders [30].

Likewise, the observed inverse relations of the BCAAs, and their derivatives, with SELENOP might reflect associations that are mediated by other compounds, such as pro-inflammatory cytokines, in turn negatively affecting SELENOP levels [11, 31].

The inverse association between the unknown metabolite X-12063 and SELENOP levels is intriguing since X-12063 has previously shown a positive association with IR and glucose intolerance [32, 33], and in a recent study it emerged as a novel marker in predicting the progression of T2DM, in normoglycaemic individuals, years before the diabetes onset [34]. In line with these observations, in the present study too the unknown X-12063 metabolite was associated with a higher likelihood of having T2DM.

Also the final oxidation product of purine metabolism, urate, displayed an inverse relation to SELENOP levels. Uric acid has shown pro-oxidant properties and has been proposed as a candidate marker of T2DM, MetS and atherosclerotic vascular disease [35]. As with the above mentioned metabolites, also the inverse association of urate with SELENOP might suggest a role of SELENOP in presaging early metabolic abnormalities on the route to diabetes and metabolic-related disorders; or lower SELENOP levels in response to these metabolic alterations, as discussed above. It should be noted however that glucose,

Table 3 Multivariable-adjusted odds ratios (OR) and 95% confidence intervals (CI) for the MetS and prevalent diabetes per 1 standard deviation increase of selected metabolites related to SELENOP levels

Metabolite	Metabolic syndrome[a] OR (95% CI)	Prevalent diabetes[b] OR (95% CI)
N (No/yes)	492/340	753/78
Amino acid		
Isoleucine	1.28 (1.04–1.60)	1.96 (1.41–2.73)
Leucine	1.06 (0.86–1.31)	1.62 (1.15–2.28)
Valine	1.05 (0.87–1.27)	1.94 (1.45–2.60)
Alanine	1.09 (0.89–1.33)	1.57 (1.17–2.11)
Glutamate	1.17 (0.95–1.45)	1.00 (0.74–1.36)
Lipid		
Caprate (10:0)	1.12 (0.86–1.47)	0.73 (0.45–1.17)
Nonadecanoate (19:0)	0.72 (0.53–0.97)	1.20 (0.74–1.95)
Arachidate (20:0)	0.98 (0.76–1.28)	1.05 (0.67–1.62)
2-oleoyl-GPC (18:1)	1.11 (0.86–1.44)	1.18 (0.77–1.80)
2-linoleoyl-GPC (18:2)	0.68 (0.51–0.90)	0.88 (0.52–1.49)
1-linolenoyl-GPC (18:3)	1.27 (0.99–1.62)	1.08 (0.67–1.74)
1-dihomo-linoleoyl-GPC (20:2)	0.87 (0.68–1.11)	0.81 (0.52–1.25)
Dodecanedioate	0.91 (0.70–1.19)	1.35 (0.90–2.03)
Sebacate (decanedioate)	0.99 (0.75–1.29)	1.03 (0.66–1.60)
Hexadecanedioate	0.87 (0.63–1.20)	0.94 (0.56–1.58)
Octadecanedioate	1.09 (0.86–1.39)	0.61 (0.38–0.97)
Docosadienoate (22:2n6)	1.33 (1.01–1.75)	1.54 (0.98–2.42)
Unknown		
X-11538	1.00 (0.78–1.29)	1.41 (1.00–1.99)
X-12063	1.10 (0.91–1.34)	1.29 (0.97–1.71)
X-16137	0.88 (0.73–1.07)	1.09 (0.84–1.42)
X-17145	0.87 (0.72–1.05)	0.88 (0.63–1.22)
Nucleotide		
Urate	1.04 (0.83–1.29)	0.93 (0.66–1.31)
Peptide		
Gamma-glutamyl-leucine	1.31 (1.05–1.64)	1.52 (1.13–2.05)
N-acetylcarnosine	1.09 (0.88–1.36)	0.61 (0.41–0.91)

$N = 831$ due to one missing value in triglycerides, total-cholesterol, and HDL-cholesterol levels.

[a]Metabolic syndrome adjusted for age, sex, education level, smoking status, physical activity, body mass index, alcohol consumption, batch number, and mutually adjusted for the other metabolites

[b]Prevalent diabetes additionally adjusted for prevalent hypertension, triglycerides, total-cholesterol and HDL-cholesterol

suggested to increase the expression of SELENOP [2], was not associated with circulating SELENOP levels. However, the LC/MS-based platform used in the present study cannot detect glucose independently from other hexoses, such as fructose, mannose, galactose, allose, and altrose [36], thus limiting our ability to corroborate the present findings.

The dipeptide gamma-glutamyl-leucine displayed inverse associations with SELENOP. Prior evidence suggests that dipeptide gamma-glutamyl-leucine serves as a biomarker for NAFLD and non-alcoholic steatohepatitis [37].

In a previous study, we observed a relation between SELENOP, MRI-defined liver signal intensity, a proxy for liver fat content, and FLD [5], which may point out to some physiological effects of the protein in modulating oxidative stress and inflammatory response [38]. Interestingly, in prior analyses from our group, dipeptide gamma-glutamyl-leucine has been linked to both liver signal intensity and FLD [18]. However, we have to acknowledge that the higher inflammatory status in participants with higher liver signal intensity, or FLD, may result in a lower SELENOP expression as a consequence of metabolic alterations.

Overall, 12 lipid compounds displayed positive associations with circulating SELENOP concentrations. The observed positive relations of SELENOP with several lipids with varying chain lengths may likely mirror diet quality and some of these lipids (caprate, nonadecanoate, arachidate, dodecanedioate, sebacate, octadecanedioate), which may reflect a vegetable-rich diet, have been related to a better metabolic profile [39–42]. Interestingly, we have previously reported a dietary pattern characterized by high intake of fruit and vegetables to be positively associated with SELENOP and inversely related to the MetS, prevalent diabetes, and lower fat depots [43]. On the other hand, considering the positive correlation of circulating SELENOP with HDL-cholesterol levels observed in the present and other studies [7, 44], and considering that some lipids showed inverse associations with the probability of having the MetS (nonadecanoate and linoleoyl-GPC), we hypothesized that the antioxidant properties of SELENOP could have been in part explained via a link with increased HDL-cholesterol levels. However, none of the SELENOP-related lipid metabolites explained the observed associations with HDL-cholesterol. Yet, the finding that linoleoyl-GPC was positively related to SELENOP levels, and in turn inversely associated with MetS, deserves further investigations. Interestingly, lower plasma linoleoyl-GPC levels have been associated with impaired glucose tolerance and T2DM [33, 45], and suggested by the same authors as an early indicator of dysglycemia and IR [45]. Also, in the present study, we observed a trend towards lower linoleoyl-GPC in participants with prevalent T2DM which, though non-significant, may land some support to these previous findings and help to further disentangle the link between SELENOP and human health. Interestingly, Ferrarini and colleagues explained that under an IR state more free fatty acids are released into the Krebs cycle, oxidized at a faster rate, and thereby negatively affecting linoleoyl-GPC levels [45]. At the same time, IR and oxidative stress—hallmarks of both

T2DM and MetS - lead to increased synthesis of glutathione, continuously depleted at advanced states, whose by products on one side lead to IR and T2DM progression, and on the other directly feed the Krebs cycle by further increasing free fatty acid oxidation and circulation, thus further reducing linoleoyl-GPC levels [45]. Putting this cycle in the context of SELENOP antioxidant function, we may speculate that increased SELENOP in the presence of glutathione, even at reduced levels, may serve to protect plasma membrane from oxidative damage [46].

Nevertheless, due to the cross-sectional design of the present study the mechanism(s) could not be characterized and any potential inference about the observed associations is tentative. Besides, we tried to focus the interpretation of our findings mainly on glucose metabolism and oxidative stress, although other metabolites, likely reflecting different pathways, emerged in our analysis.

Strengths and limitations

Strength of the present study include the assessment of serum metabolites using a non-targeted approach, which provided a comprehensive analysis of all the measurable compounds and bear the potential for novel pathways discovery.

The following limitations merit consideration. The cross-sectional study design can raise hypotheses for future investigations, but it does not allow causal inferences. We cannot completely rule out model misspecification or residual confounding, though we adjust the statistical models for several covariates. Yet, we did not have available measure of insulin resistance, which might have increased our understanding of the observed associations. The chemical identity of several analyzed metabolites is, so far, still unknown, and we relied on one single measurement of metabolites, thus we could not address their reliability over time. Furthermore, we excluded from the analyses 73 metabolites with more than 80% missing data, thus precluding the possibility to identify additional potential disease markers and new knowledge about the function of SELENOP. However, our choice was substantiated by the large error terms observed when such metabolites where used in the analyses, particularly for those with more than 90% missing, thus limiting our ability to detect reliable associations. We did not have direct Se measures available though SELENOP is considered a sensitive biomarker for Se status, particularly in populations with low-to-moderate selenium status as in Germany [47]. Lastly, the applicability of our observations to other age groups and ethnicities is unknown since our sample comprises adult participants from Northern Germany.

In conclusion, using an untargeted metabolomics approach, we observed that higher serum SELENOP levels were associated with an overall healthy metabolomics profile characterized by lower concentrations of branched-chain and other amino acids as well as an unknown compound previously related to IR and several lipids with different chains length. These associations may provide novel biological insights relevant to disentangle the role of SELENOP in human health. Yet, the present findings should be considered preliminary and as such re-evaluated and validated in larger studies before further conclusions on the biological mechanisms linked to SELENOP can be drawn.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study procedures have been approved by the Ethics Committee of the Medical Faculty of the University of Kiel, Germany.

Informed consent Informed consent was obtained from all individual participants included in the study.

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