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ABSTRACT

Background: Higher meat intake has been associated with adverse health outcomes, including cardiovascular disease (CVD). This study investigated plasma metabolites associated with meat intake and their relation with cardiometabolic biomarkers, subclinical CVD markers, and incident CVD.

Methods: Associations between self-reported meat intake and 1272 plasma metabolites were investigated in the SCAPIS cohort (n = 8,819; ages 50–64). Meat-associated metabolites were further examined for relation with subclinical CVD markers in the POEM cohort (n = 502; age 50) and incident CVD in the EpiHealth cohort (n = 2,278; ages 45–75; 107 incident cases over 9.6 years follow-up). Meat intake was categorized into white, unprocessed red, and processed red meat. Linear regression analyzed associations between meat intake, metabolites and cardiometabolic biomarkers, and subclinical CVD markers, while Cox models evaluated association between meat-associated metabolites and incident CVD.

Results: After correction for multiple testing, 458, 368, and 403 metabolites were associated with white, unprocessed red, and processed red meat, respectively. Processed red meat-associated metabolites were associated with higher levels of fasting insulin, hemoglobin A1c, and lipoprotein(a), and were inversely associated with maximal oxygen consumption. Two metabolites, 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2) (hazard ratios (HR: 1.32; 95 % CI: 1.08, 1.62)) and glutamine degradant (HR: 1.35; 95 % CI: 1.07, 1.72), that were inversely associated with intake of all meat types, were also associated with a higher risk of incident CVD.

Conclusions: This study provides comprehensive analysis of self-reported meat intake and plasma metabolites. The findings may enhance our understanding of the relationship between meat intake and CVD, and provide insights into underlying mechanisms.

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1. Introduction

Meat is an important dietary component as it provides essential nutrients including protein and micronutrients such as iron, zinc and vitamin B12 [1]. Nevertheless, epidemiological studies have shown that high meat intake is associated with an increased risk of cardiovascular disease (CVD) incident and its risk factors, including blood lipids, insulin, glucose, hemoglobin A1c (HbA1c), C-reactive protein and insulin sensitivity [2–4]. However, significant variations in these associations have been observed across studies [5,6]. The mechanisms by which meat intake may influence CVD risk are complex. Heme iron content in red meat, along with saturated fats, processing methods involving sodium and nitrates which are precursors of nitrosamines, and high temperature heating, contribute to advanced glycation and other Maillard products, which may contribute to the development of CVD [7–9]. White meat, such as chicken and turkey, is a lower-fat and lower-iron alternative to red meat and may protect against CVD, although evidence supporting the protective nature of white meat is conflicting [10]. Thus, understanding the mechanisms through which meat intake may increase the risk of CVD remains challenging and requires further exploration.

Recent advances in high-throughput metabolomic profiling offer a powerful approach for identifying plasma metabolites associated with habitual meat intake and their clinical relevance [11,12]. Metabolomic profiles enable a more objective assessment of metabolic responses, addressing the systematic and random measurement errors inherent in self-reported dietary methods, such as food frequency questionnaires (FFQs) [13]. Furthermore, sample heterogeneity, which arises from factors such as variations in meat consumption across populations, differences in study designs, geographical and dietary influences, and inter-individual molecular variation, play a significant role in the evaluation of the meat-metabolite associations [14].

Previous studies have indicated an association between meat intake and plasma metabolites. For instance, a recent study based on a type 2 diabetes case-control study (n = 403) and replication in a population-based sample of 4,457 individuals identified associations of processed meat with acylcarnitines and a piperine metabolite [15]. Similarly, the PREDIMED study (n = 1,833) found that red meat intake was linked to higher levels of 38:4 PC plasmalogen, cotinine and isoleucine, while processed red meat consumption was associated with increased levels of leucine, uric acid, and 36:5 PC plasmalogen [16]. Furthermore, in the Bavarian Food Consumption Survey (n = 294), red meat intake was associated with higher plasma levels of carnosine and pi-methylhistidine, whereas poultry intake was associated with pi-methylhistidine levels [17]. In a dietary intervention study (n = 10), chicken intake was associated with anserine, while red and processed meats were associated with carnosine and acylcarnitines such as acetylcarnitine, propionylcarnitine, and 2-methylbutyrylcarnitine [18]. However, a larger epidemiological study that accounts for other dietary components and examines the links to cardiometabolic biomarkers, subclinical CVD markers, and incident CVD could provide more robust evidence of associations between specific meat intake, related metabolites, and CVD risk.

Therefore, our primary aim was to investigate the association between self-reported white meat, unprocessed red meat, and processed red meat intake with plasma metabolites using the Swedish CArdio-Pulmonary bioImage Study (SCAPIS) cohort. As a secondary aim, we investigated the identified meat-associated metabolites in relation to cardiometabolic biomarkers in the SCAPIS cohort, their relationship with subclinical CVD markers in the POEM cohort, and incident CVD in the EpiHealth cohort.

2. Methods

2.1. Study population

The current study included data and samples from three population-based cohorts: SCAPIS, POEM, and EpiHealth. The studies were conducted in compliance with the principles of the Declaration of Helsinki and were approved by the responsible ethics committees (DNR 2023–07352-01, DNR 2009–057, DNR 2018–315), and written informed consent was obtained from all participants.

The SCAPIS is a nationwide, population-based study conducted in Sweden between 2013 and 2018. It involved 30,154 men and women aged 50–64 years, who were randomly selected from the Swedish population register and recruited from six sites. For the current analyses, we included 11,287 participants from two sites of SCAPIS, namely SCAPIS-Uppsala (n = 5,036) and SCAPIS-Malmö (n = 6,251) [19]. A total of 8,966 particiapnts were included for metabolomic analysis from the SCAPIS-Uppsala (n = 4,990) and SCAPIS-Malmö (n = 3,976). Further removed in quality control (n = 9) and excluding participants with missing covariates (n = 138) in the full model resulted in a final sample of 8,819 participants, comprising from SCAPIS-Uppsala (n = 4,906) and from SCAPIS-Malmö (n = 3,913). The flowchart of the study design is shown in Fig. S1. Further information of participants from the POEM and EpiHealth cohorts are provided in the Supplemental Method.

2.2. Dietary assessment

Dietary intake was assessed using a web-based FFQ (MiniMeal-Q), which had been previously validated for the SCAPIS cohort [20,21]. Briefly, the FFQ was self-administered, semi-quantitative, and included questions about dietary intake and portion sizes. The questions offer an optional nine-point frequency scale, ranging from “five times per day” to “one to three times per month”. To account for this variation, the questionnaire includes between 75 and 126 food items. These food items include the consumption of (1) foods, cooked dishes, and beverages; (2) energy and nutrient intake, including alcohol; (3) dietary supplements; (4) meal patterns; and (5) eating habits, such as dining out, fast food consumption, use of low-calorie products and probiotics, and the consumption of cooking fats and salt. Participants chosed from a list of predetermined food items and frequency options, including only those they consumed at least once a month [20]. During their initial visit, participants were asked to indicate how often, on average, they had consumed various foods for a month, ranging from five times a day to one to three times a month. In this study, meat intake was categorized into white meat, unprocessed red meat, and processed red meat, based on classifications made in previous Swedish studies [22,23]. White meat included chicken/other poultry; unprocessed red meat included pork, beef, lamb, game, hamburger, kebab, and minced meat dishes; and processed red meat included ham/salami for sandwiches and sausages. Meat intake (g/d) was calculated using frequency of meat intake and portion size information for all three meat types.

2.3. Plasma metabolome profiling

In the three study cohorts, plasma samples were analyzed using the same untargeted metabolomics platform from Metabolon Inc. (Durham, NC, USA) [24] to ensure consistency in the identification of metabolites. Fasting plasma samples were collected after overnight fasting and stored at −80 °C in the respective biobanks until they were sent to Metabolon for non-targeted metabolome analysis. Quality control standards for metabolome analysis have been previously described in studies [25–27]. Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) was used, employing a heated electrospray ionization (HESI-II) source and an Orbitrap mass analyzer. Four approaches were applied to maximize metabolite coverage and identification: two separate reverse-phase UPLC-MS/MS analyses in positive

ionization mode (electrospray ionization, +ESI), a reverse-phase UPLC-MS/MS analysis in negative ionization mode (-ESI), and a hydrophilic interaction UPLC-MS/MS analysis in negative ionization mode (-ESI).

Metabolites were classified using two different levels by Metabolon. The first level, metabolite class called the "super-pathway," includes broad categories such as amino acids, lipids, uncharacterized molecules, xenobiotics, cofactors and vitamins, nucleotides, carbohydrates, peptides, energy-related metabolites, and non-significant metabolites. The second level, metabolite subclass, called, "sub-pathway," refers to more specific pathway categories, with 116 distinct sub-pathway types. Further information on the untargeted metabolomics analysis workflow for metabolite identification is provided in the Supplemental Method.

2.4. Cardiometabolic biomarker measures

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using standard laboratory methods. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [28]. Fasting plasma samples were analyzed on the Alinity C system for apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), and lipoprotein(a) (Lp(a)), while insulin was assessed using the Alinity I kit. Plasma glucose was determined by the hexokinase method. Hemoglobin A1c (HbA1c) was measured in SCAPIS-Uppsala using capillary electrophoresis and in SCAPIS-Malmö via turbidimetry. C-reactive protein (CRP) was measured using the immunoturbidimetric method. Blood pressure was measured using an Omron M10-IT device.

2.5. Covariates

Self-reported covariates included demographic and lifestyle factors. A detailed description of the covariates is provided in the Supplemental Method.

2.6. Statistical analysis

Individual-level data from the SCAPIS-Uppsala and SCAPIS-Malmö cohorts were combined for analysis. The data was checked for missing values, outliers, and statistical distributions. Missing values were addressed using complete case analysis. The characteristics of study participants were presented based on their tertiles of meat intake, using means and standard deviations (SD) for continuous variables and number (%) for categorical variables (Table 1). For the descriptive table, the measures of white meat, unprocessed red meat, and processed red meat intake were combined into one variable as total meat intake, which was divided into tertiles. Pearson correlation coefficients were calculated to examine the relationships between the intake of the three meat

Table 1
Characteristics of study participants based on the first and third tertiles of total meat intake in the SCAPIS-Uppsala (T1, $n = 1{,}546$; T3, $n = 1{,}684$) and SCAPIS-Malmö (T1, $n = 1{,}402$; T3, $n = 1{,}274$) cohorts.

SCAPIS-Uppsala				SCAPIS-Malmö		
	T1	T3	Total	T1	T3	Total
	(n = 1,546)	(n = 1,684)	(n = 4,906)	(n = 1,402)	(n = 1,274)	(n = 3,913)
Age, years	58.2 (4.3)	57.0 (4.4)	57.7 (4.4)	57.9 (4.3)	56.9 (4.2)	57.5 (4.3)
Sex: male	470 (30.4)	1155 (68.6)	2385 (48.6)	419 (29.9)	859 (67.4)	1866 (47.7)
BMI, kg/m^2	26.2 (4.2)	28.0 (4.5)	27.0 (4.4)	26.3 (4.3)	28.4 (4.8)	27.2 (4.5)
Energy total, kcal/d	1518.5 (578.5)	1988.2 (730.5)	1731.6 (677.5)	1501.8 (638.5)	2034.3 (823.3)	1730.2 (749.1)
Fibre intake, g/d	21.1 (11.2)	20.7 (10.6)	20.4 (10.7)	20.4 (11.9)	20.7 (11.4)	20.0 (11.3)
Carbohydrate, g/d	165.8 (70.3)	207.0 (92.4)	184.6 (83.47)	165.3 (83.7)	212.7 (106.7)	186.0 (95.6)
Fat, g/d	60.2 (27.9)	81.4 (33.5)	69.7 (31.0)	59.7 (28.7)	83.2 (36.4)	69.6 (33.3)
Protein, g/d	58.6 (21.3)	83.0 (27.6)	69.7 (26.0)	56.7 (22.3)	83.7 (28.8)	68.1 (27.1)
Fish intake, g/d	28.9 (22.1)	42.4 (27.4)	35.5 (24.9)	26.3 (22.7)	39.5 (27.26)	32.4 (24.9)
Egg intake, g/d	20.7 (33.2)	25.1 (38.6)	22.2 (33.9)	21.3 (37.2)	23.5 (31.9)	21.7 (32.4)
Whole grain intake, g/d	44.8 (44.3)	45.4 (43.5)	44.3 (44.0)	41.2 (48.3)	39.4 (40.8)	39.5 (43.4)
Fruit,vegetable intake, g/d	361.4 (241.0)	287.4 (202.0)	318.1 (218.6)	368.1 (253.6)	310.2 (228.2)	331.6 (237.1)
Alcohol intake, g/d	5.5 (5.5)	8.2 (6.8)	6.9 (6.2)	5.3 (5.7)	8.6 (7.9)	7.0 (6.8)
Physical activity, n (%)						
Never	368 (25.4)	461 (29.7)	1233 (26.9)	415 (30.0)	420 (33.7)	1205 (31.3)
Not regular	310 (21.4)	313 (20.2)	980 (21.3)	314 (22.7)	287 (23.1)	879 (22.9)
1–2 times/week	307 (21.2)	325 (21.0)	981 (21.4)	246 (17.8)	207 (16.6)	703 (18.3)
2–3 times/week	263 (18.2)	287 (18.5)	842 (18.3)	235 (17.0)	177 (14.2)	587 (15.3)
>3 times/week	198 (13.7)	165 (10.6)	555 (12.1)	172 (12.4)	154 (12.4)	470 (12.2)
Regular smokers, n (%)	83 (13.0)	73 (12.3)	223 (11.8)	125 (16.5)	123 (17.4)	389 (18.2)
White meat, g/d	11.8 (9.8)	33.8 (20.7)	22.7 (17.5)	11.8 (9.7)	35.7 (24.8)	22.9 (19.7)
Unprocessed red meat, g/d	20.2 (12.9)	83.5 (32.9)	50.7 (34.1)	18.9 (12.2)	82.3 (38.1)	47.6 (35.9)
Processed red meat, g/d	8.2 (8.1)	36.3 (23.6)	21.8 (19.9)	9.3 (9.2)	37.9 (27.6)	22.1 (21.7)
HDL-C, mmol/L	1.6 (0.4)	1.4 (0.4)	1.5 (0.4)	1.8 (0.6)	1.5 (0.5)	1.7 (0.5)
LDL-C, mmol/L	3.5 (0.9)	3.5 (0.9)	3.5 (0.9)	3.6 (1.0)	3.6 (1.0)	3.6 (1.0)
Total cholesterol, mmol/L	5.7 (1.0)	5.6 (1.1)	5.7 (1.1)	5.5 (1.0)	5.4 (1.0)	5.4 (1.0)
Triglycerides, mmol/L	1.2 (0.6)	1.4 (1.0)	1.3 (0.8)	1.2 (0.7)	1.4 (0.9)	1.3 (0.8)
Insulin, mIE/L	7.0 (13.9)	8.9 (18.2)	7.9 (16.1)	6.8 (5.3)	9.7 (45.6)	8.0 (26.8)
Glucose, mmol/L	5.8 (1.0)	6.0 (1.2)	5.9 (1.1)	5.8 (1.2)	6.0 (1.4)	5.9 (1.3)
HbA1c, mmol/mol	35.9 (5.6)	36.7 (7.1)	36.2 (6.1)	37.1 (6.5)	37.7 (7.9)	37.2 (7.1)
SBP, mmHg	124.1 (16.6)	126.4 (15.0)	125.1 (15.9)	121.6 (17.5)	123.8 (15.5)	122.5 (16.4)
DBP, mmHg	76.6 (10.1)	77.5 (9.6)	77.0 (9.8)	74.2 (9.9)	75.4 (9.3)	74.8 (9.6)
CRP, μmol/L	2.1 (3.8)	2.4 (4.5)	2.2 (4.0)	2.1 (3.4)	2.5 (4.4)	2.3 (4.1)
Hypertension medication[b]	273 (18.8)	320 (20.4)	889 (19.2)	270 (19.7)	275 (22.2)	805 (21.0)
Cholesterol medication[b]	111 (7.6)	133 (8.5)	358 (7.7)	106 (7.7)	119 (9.6)	331 (8.6)
Diabetes medication[b]	47 (3.2)	80 (5.1)	168 (3.6)	65 (4.7)	62 (5.0)	173 (4.5)

Values are mean (SD) for continuous variables or number (%) for categorical variables. T, tertiles, T1 = low consumers of all meat types, T3 = higher consumers of all meat types, BMI, body mass index, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein. [b]Self-reported medication use of the last 2 weeks.

types. Plasma metabolites were log-transformed and standardized to a mean of 0 and SD of 1. The biomarkers TG, CRP, Lp(a) and insulin had skewed distributions, and thus were log-transformed prior to analysis. We analyzed association of meat types with metabolites using linear regression analysis. All estimates from the linear regression analyses were reported per 20-g-per-day increase in meat intake, which corresponds to approximately a 1 SD increase for white and processed red meat, and a 0.5 SD increase for unprocessed red meat. Two sets of regression analyses were performed. First, the basic model was adjusted for age, sex, metabolomic delivery batch, total energy intake, intake of fibre, whole grain, fruits and vegetables, as well as alcohol consumption, physical activity, education, smoking status, history of hypertension, and medication use for hypertension, cholesterol, and diabetes. Second, the full model included the same covariates as the basic model, with additional adjustment for body mass index (BMI). In both models, the q-values were derived using the Benjamini-Hochberg procedure to control for the false discovery rate (FDR) across all statistical tests performed [29]. The multiple testing correction was applied, and FDR-adjusted *p-values (q-values)* were estimated, with a threshold set <0.05.

Ordinal (proportional odds) regression was used as a sensitivity analysis for significant findings in the associations between meat intake and metabolites from the full model. Further sensitivity analyses were performed with additional adjustments for other animal-source foods, such as fish and eggs; mutual adjustment for different meat types; and an adjustment for fibre intake, accounting for potential confounding factors while excluding major food sources of fibre. An additional sensitivity analysis was conducted by excluding participants with prevalent CVD and diabetes mellitus (DM). Linear regression models were used to analyze the association of the top 20 meat-associated metabolites with cardiometabolic biomarkers adjusted for age, sex, metabolomic delivery batch, total energy intake, alcohol intake, smoking, physical activity, and education. Similarly, the top 20 meat-associated metabolites in relation to subclinical CVD markers in POEM were examined using linear regression analysis, adjusted for sex, BMI, waist-hip ratio, smoking status, systolic blood pressure (SBP) and diastolic blood preesure (DBP), fasting blood glucose, creatinine level, total cholesterol, HDL-C, insulin, history of diabetes, cholesterol-lowering drugs, medication for diabetes and other lipid lowering agents. Furthermore, summary results regarding metabolite-incident CVD were extracted from previously published finding for the EpiHealth study, with details on statistical analysis and confounding variables reported previously [26].

Metabolic enrichment pathways analyses were performed based on the ranked association *q-values* of the significant metabolites from fully adjusted model results for each meat type and its associated metabolites, using the fast gene set enrichment analysis (fgsea) package [30] v1.19.2 package in R version 4.3.2 for both positively and negatively associated metabolites. Statistical analyses were performed using Stata version 18.0 and visualizations were created using ComplexHeatmap package in R version 4.3.2 [31].

3. Results

3.1. Participant characteristics

Characteristics of the study participants are presented as the extreme tertiles (tertile 1 (T1) vs tertile 3 (T3)) of total meat intake (Table 1). We observed higher mean (SD) of BMI, total energy intake, and alcohol intake among participants with higher total meat intake at both study sites. **Tables S1, S2, and S3** provide the characteristics of the study participants based on the extreme tertiles of white meat, unprocessed red meat, and processed red meat intake, respectively. Pearson correlation coefficients were calculated to examine the relationships between the intake of the three meat types: white meat intake was positively correlated with unprocessed red meat intake ($r = 0.35$, p-value <0.0001) and processed red meat intake ($r = 0.12$, p-value <0.0001), and unprocessed red meat intake was also positively correlated with processed red meat intake ($r = 0.38$, p-value <0.0001).

3.2. Metabolites associated with meat intake

We analyzed the association of white meat, unprocessed red meat, and processed red meat intake with plasma metabolites. In the basic model, 477 metabolites were associated with white meat **(Table S4)**, 489 with unprocessed red meat **(Table S5)**, and 386 with processed red meat intake **(Tables S6)**, respectively (q-value<0.05).

In the fully adjusted model, white meat intake was associated with 458 metabolites, including 130 positive and 328 negative associations **(Table S7)**. Fig. 1 shows the results for the top 20 metabolites associated with each meat type in the full model, selected based on the lowest q-values. Among the top findings, white meat intake showed a strong positive association with 1-methyl-5-imidazolelactate ($\beta = 0.08$; SE = 0.004 per 20 g of white meat intake; q-value $= 5.16 \times 10^{-78}$), while the strongest negative association was observed with glutamine degradant ($\beta = -0.03$; SE = 0.003 per 20 g of white meat intake; *q-value* $= 4.05 \times 10^{-42}$) **(**Fig. 1, **Table S7)**.

In the fully adjusted model, unprocessed red meat intake was associated with 403 metabolites, comprising 165 positive and 238 negative associations **(Table S8)**. Among the top findings, we observed that unprocessed red meat intake had a strong positive association with 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4) ($\beta = 0.02$; SE = 0.001 per 20 g of unprocessed red meat intake; *q-value* $= 1.64 \times 10^{-44}$), while a strong negative association was observed for glutamine degradant metabolite ($\beta = -0.02$; SE = 0.002 per 20 g of unprocessed red meat intake; *q-value* $= 1.13 \times 10^{-33}$) **(**Fig. 1, **Table S8)**. Similarly, processed red meat intake was associated with 368 metabolites, consisting of 168 positive and 200 negative associations **(Table S9)**. Strongest positive and negative associations were observed for the metabolites 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4) ($\beta = 0.02$; SE = 0.002 per 20 g of processed red meat intake; *q-value* $= 1.54 \times 10^{-33}$) and 2,6-dihydroxybenzoic acid ($\beta = -0.04$; SE = 0.004 per 20 g of processed red meat intake; *q-value* $= 1.02 \times 10^{-25}$), respectively (Fig. 1, **Table S9)**.

Fig. 2 shows the plasma metabolites associated with the intake of white meat, unprocessed red meat, and processed red meat, categorized by metabolite classes. Metabolites positively associated with white meat intake primarily belonged to the metabolite classes amino acids and lipids. However, for unprocessed red meat, the positively associated metabolites primarily belonged to lipids and xenobiotics, while for processed red meat they belonged to lipids, xenobiotics, and uncharacterized molecules. The specific and overlapping metabolites identified in association with meat exposures are found in **Fig. S2**. There were 142 metabolites unique to white meat, 63 unique to unprocessed red meat, and 109 unique to processed red meat, while 145 metabolites overlapped among the three types.

The associations from the fully adjusted model were tested in an ordinal (proportional odds) regression as a sensitivity analysis for our significant findings and overall supports the conclusion from the main analysis in linear regression. The Spearman correlation between p-values from the main analysis and sensitivity analysis was 0.92 for white meat, 0.89 for unprocessed red meat and 0.91 for processed red meat (**Fig. S3**, **Table S10**).

We also conducted additional sensitivity analyses, adjusting for fibre intake while excluding major food sources **(Tables S11–S13)**, as well as fish and egg intake **(Tables S14–S16)**. **Further analyses included** mutual adjustments for other meat types **(Tables S17–S19)** and exclusion of participants with prevalent CVD and DM **(Tables S20–S22)**. We observed that mutual adjustment reduced the number of meat-associated metabolites compared to the associations with each meat type, with a further attenuation observed after adjusting for fish and egg intake. In contrast, the number of associated metabolites increased with fibre intake and after excluding participants with prevalent CVD and DM. After mutual adjustments, we identified 362, 201, and 361 metabolites were associated with white meat, unprocessed red meat, and

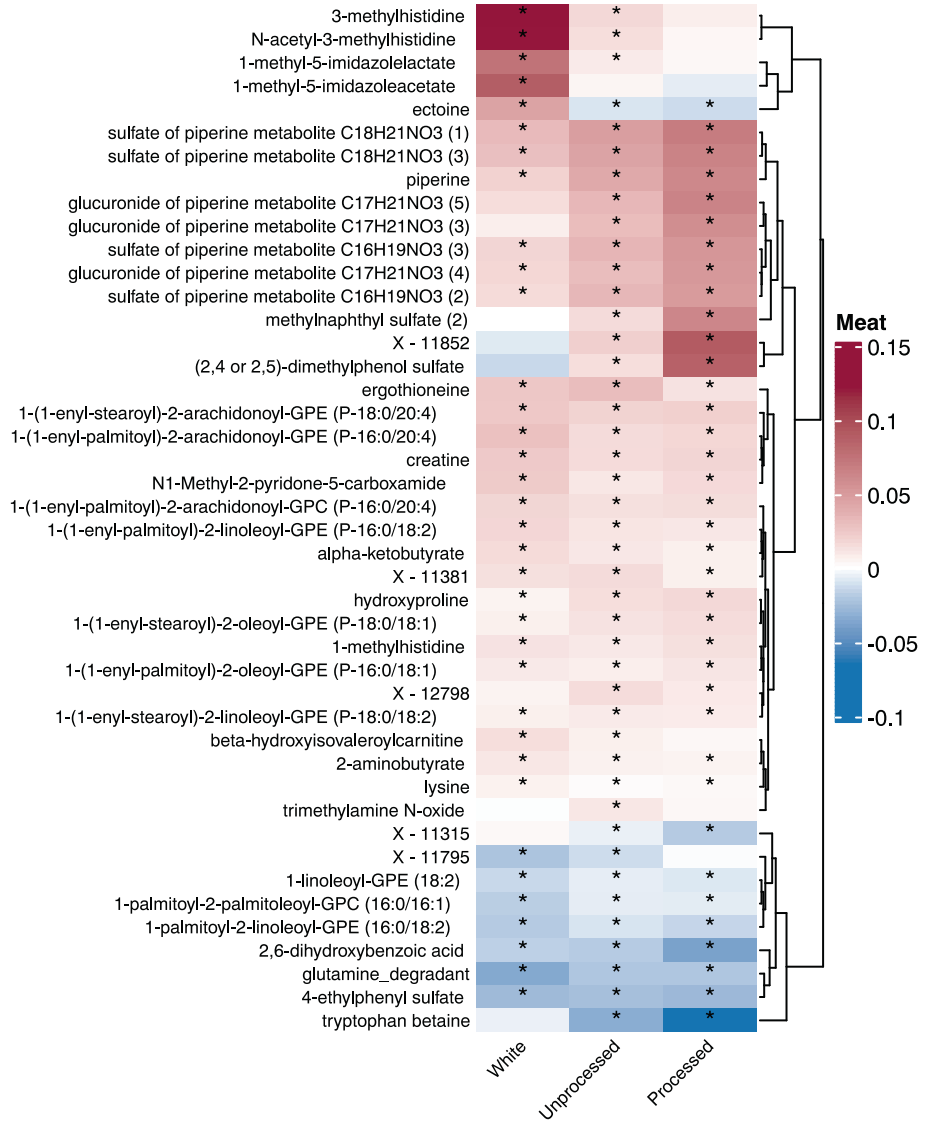

Fig. 1. Heatmap illustrating the associations of meat intake of white, unprocessed red meat and processed red meat with top 20 plasma metabolites. Linear regression coefficients (β) were adjusted for age, sex, batch effect, total energy intake, fibre intake, whole grain intake, fruit and vegetable intake, alcohol intake, physical activity, education, smoking history, hypertension medication, cholesterol medication, and diabetes medication. Asterisk (*) indicates statistical significance, as determined by false discovery rate–adjusted *p-values (q-values)* < *0.05.* Metabolites with different numbers at the end of the name (e.g., glucuronide of piperine metabolite C17H21NO3 (5) and C17H21NO3 (3)) represent distinct isomers or metabolites within the same metabolic pathway. The use of a slash (/) distinguishes between for example P-18:0 (sn1) and 20:4 (sn2) for example, with the metabolite identified based on accurate mass spectrometry data. **sn1**: The fatty acid attached to the first position of the glycerol molecule; **sn2**: The fatty acid attached to the second position of the glycerol molecule. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

processed red meat, respectively. Further adjustments for fish and egg intake revealed associations with 399, 365, and 348 metabolites for white meat, unprocessed red meat, and processed red meat, respectively. When additionally adjusting for fibre intake, the number of associated metabolites increased to 476, 393, and 375 for white meat, unprocessed red meat, and processed red meat, respectively. Moreover, after excluding participants with prevalent CVD and DM, we observed 416, 380, and 354 metabolites associated with white meat, unprocessed red meat, and processed red meat, respectively.

We also compared our findings with a recently published review article on metabolites associated with meat intake, which identified nine robust metabolites associated with different types of meat and reported them in both intervention and observational studies [32] (Fig. S4, Table 2). Similar to the previous findings, in the present study, we observed white meat intake to be associated with 3-methylhistidine and carnitine, the latter of which was reported as *O*-acetyl-L-carnitine in the previous study. We also detected associations of both processed and unprocessed red meat with carnitine and carnosine. Furthermore, our analysis found that unprocessed red meat, but not processed red meat, was positively associated with trimethylamine *N*-oxide (TMAO).

3.3. Pathway Enrichment Analysis of Metabolites Associated with Meat Intake

Fig. 3 and **Tables S23-S31** depict the associations between meat-associated metabolites and their specific metabolic pathways. We observed positive enrichment of plasmalogen metabolic pathway for metabolites associated with the three meat types **(Tables S25, Table S28, Table S31)**. Moreover, white meat-associated metabolites were positively enriched for leucine, isoleucine, and valine metabolism (Fig. 3, **Table S25**), while unprocessed red meat was enriched for histidine metabolism (**Table S28**). Metabolites associated with processed

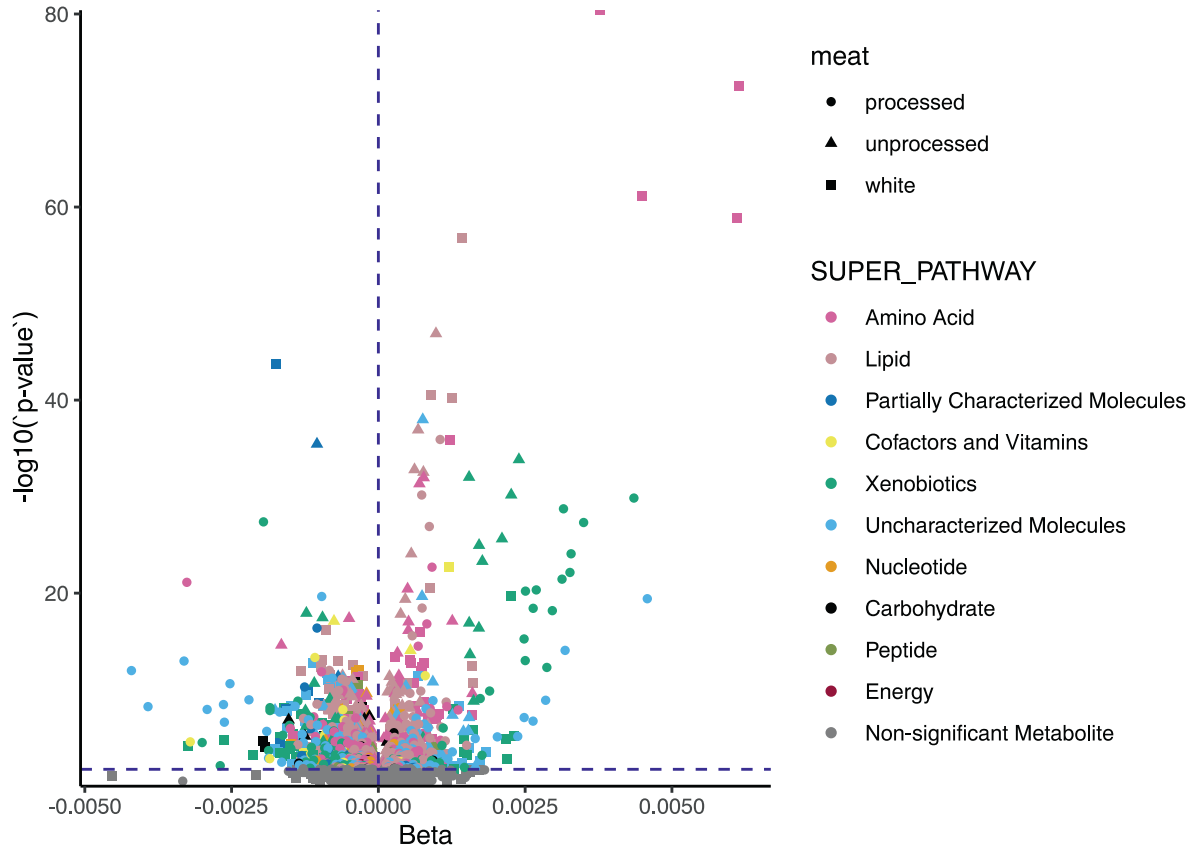

Fig. 2. Volcano plot showing the association of meat intake of white, unprocessed red meat and processed red meat plasma metabolites relative to their metabolite classes. Points above the red horizontal dashed line indicate statistical significance based on *q-values* < *0.05*. X-axis shows the meat-metabolites association beta coefficients and y-axis shows the -$\log_{10}$ *p*-values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Meat intake in association with most frequently identified meat-associated metabolites in participants from the SCAPIS-Uppsala and SCAPIS-Malmö cohorts.

		White meat				Unprocessed red meat			Processed red meat		
Metabolites	Previous findings	n	Beta	SE	*q-value*	Beta	SE	*q-value*	Beta	SE	*q-value*
3-methylhistidine	Chicken/Poultry (+)	8193	0.12	0.01	1.9e-70*	0.02	0.004	1.00E-04*	0.01	0.01	4.1E-01
Acetyl-carnitine (C2)	Poultry (+), Processed meat(+), red meat (+)	8193	−0.002	0.001	3.5E-01	0.003	0.001	6.0E-05*	0.01	0.001	8.8E-05*
Carnitine	Red meat (+)	8193	0.003	0.003	3.0E-03*	0.005	0.001	5.3E-10*	0.004	0.001	4.4E-03*
Carnosine	Chicken/Poultry (+), red meat (+)	7771	0.01	0.01	9.4E-02	0.02	0.003	2.6E-07*	0.02	0.005	1.7E-04*
Creatine	Total meat (+)	8193	0.02	0.02	1.E-36*	0.02	0.001	1.5E-30*	0.02	0.002	2.9E-21*
Creatinine	Total meat (+)	8193	−0.0004	0.00	6.7E-01	0.000	0.000	3.4E-01	0.001	0.001	5.7E-01
Glutamine	Total meat (+)	7800	0.01	0.01	3.5E-01	0.01	0.007	3.9E-01	0.01	0.01	7.1E-01
Trimethylamine N-oxide	Red meat (+)	8193	−0.001	0.003	9.1E-01	0.011	0.002	1.5E-07*	0.004	0.003	4.0E-01
Hydroxyproline	Total meat (+)	8193	0.002	0.002	2.7E-02	0.01	0.001	5.8E-30*	0.02	0.002	9.8E-16*

[a]Previous findings were based on a recent review of metabolites associated with meat intake (https://pmc.ncbi.nlm.nih.gov/articles/PMC8634495/).
The '+' denotes metabolites positively linked to different meat types.
* Asterisk () indicates statistical significance (False Discovery Rate < *0.05*).

red meat showed positive enrichment in benzoate metabolism and fatty acid metabolism (acyl carnitine, medium chain) pathways (**Table S31**).

3.4. Associations of meat-associated metabolites with cardiometabolic biomarkers, sublinical CVD markers and incident CVD

Next, we selected the top 20 metabolites associated with each meat type (based on *q*-values) and analyzed their association with cardiometabolic biomarkers **(Table S32-S34)**, sublinical CVD markers (**Table S35-S37**), and incident CVD (**Table S38-S40**).

We observed that metabolites positively associated with all three meat types were also positively associated with ApoA1 and CRP **(**Fig. 4**,** Fig. S5**, Table S32-S34)**. Three metabolites, 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4), 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4), and 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P 16:0/20:4), were positively associated with intima-media thickness (IMT) of the carotid artery. We also observed that the metabolite 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4) was inversely associated with intima-media grayscale median (IMGSM), endothelium dependent vasodilation (EIDV), and endothelium dependent

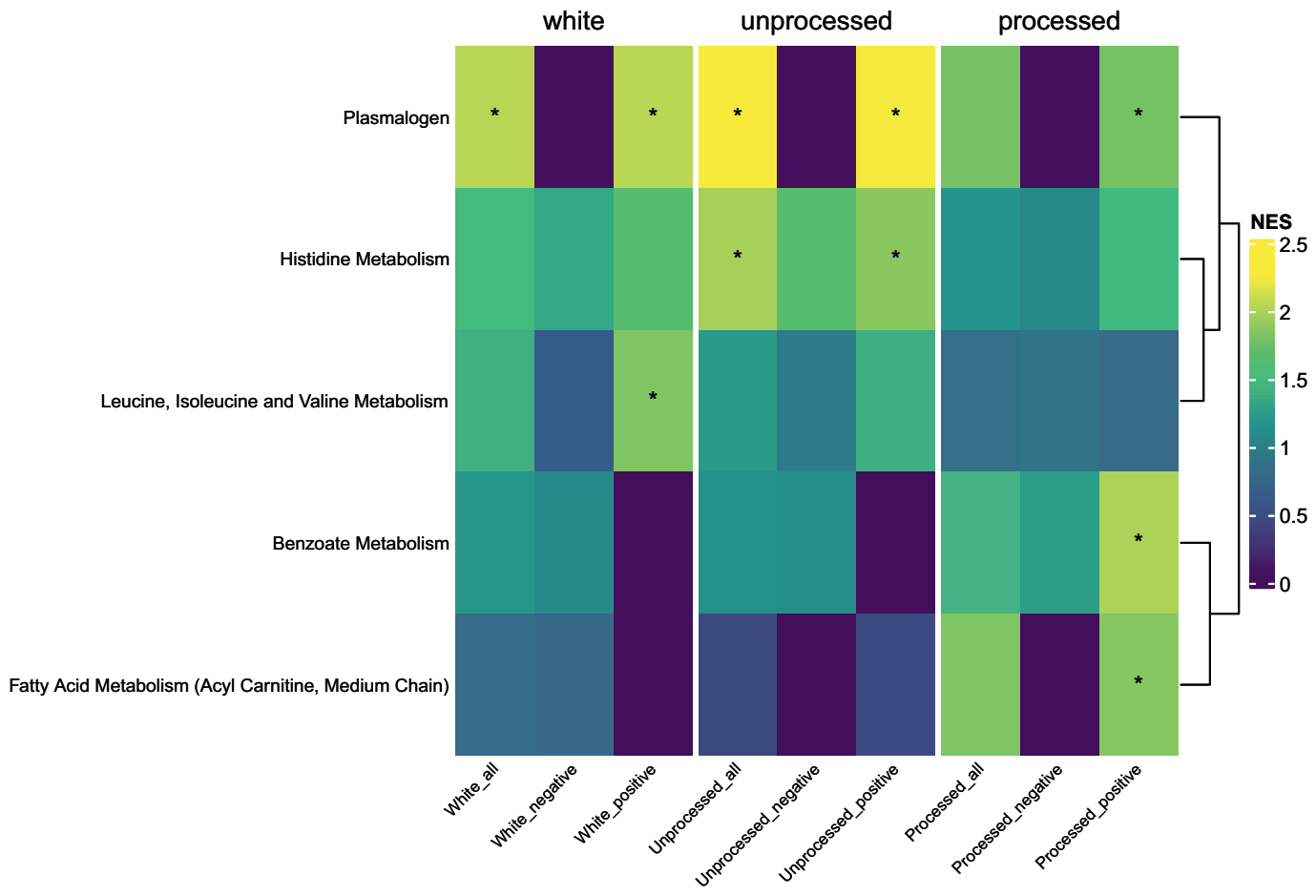

Fig. 3. Gene enrichment analysis of meat-related metabolites. The heatmap shows the enriched metabolic sub-pathways in the associations between meat intake of white meat, unprocessed red meat and processed red meat-associated metabolites stratified by the direction of the associations. X-axis represents meat-metabolites associations and y-axis represents different metabolic pathways. White_ all, pathway enrichment analysis of metabolites associated with white meat intake (both negatively and positively); white_negative, pathway enrichment analysis of metabolites negatively associated with white meat intake; white_positive, pathway enrichment analysis of metabolites positively associated with white meat intake; unprocessed_all, pathway enrichment analysis of metabolites associated with unprocessed red meat intake (both negatively and positively),; unprocessed_negative, pathway enrichment analysis of metabolites negatively associated with unprocessed red meat intake; unprocessed_positive, pathway enrichment analysis of metabolites positively associated with unprocessed meat intake; processed_all, pathway enrichment analysis of metabolites associated with processed meat intake (both negatively and positively); processed_negative, pathway enrichment analysis of metabolites negatively associated with processed red meat intake; processed_positive, pathway enrichment analysis of metabolites positively associated with processed red meat intake; NES, Normalized Enrichment Score. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

vasodilation (EDV) subclinical markers (Fig. 4, **Table S35-S37**).

Moreover, we found that metabolites negatively associated with all three meat types were positively associated with higher fasting insulin levels (Fig. 4, Fig. S5, **Table S32-S34**). Two metabolites including 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2) (hazard ratios (HR: 1.32; 95 % CI: 1.08, 1.62)) and glutamine degradant (HR: 1.35; 95 % CI: 1.07, 1.72) were associated with an increased risk of incident CVD (Fig. 4, **Table S38-S40**).

We found that the metabolite ectoine, which was positively associated with white meat, but negatively associated with unprocessed and processed red meat, was associated with higher pulse wave velocity (PWV) (Fig. 4, **Table S35**). Moreover, metabolites positively associated with processed red meat intake were also associated with higher levels of insulin, HbA1c and Lp(a) (Fig. 4, **Table S34**), and inversely associated with maximal oxygen consumption (VO_2 max) (Fig. 4, **Table S37**).

We observed that TMAO metabolite that was positively associated with unprocessed red meat intake (Fig. 1), was associated with higher levels of fasting insulin, HDL-C, LDL-C and glucose (Fig. 4, **Table S33**), but was not associated with subclinical CVD markers or incident CVD.

4. Discussion

In this large population-based study, we investigated the association between white, unprocessed red, and processed red meat intake and plasma metabolites. After adjusting for potential confounding factors, we identified 458 metabolites that were associated with white meat, 403 with unprocessed red meat, and 368 with processed red meat. Of these, 142 metabolites were unique to white meat, 63 to unprocessed red meat, and 109 to processed red meat, while 145 metabolites were overlapping among the three meat types. Metabolites associated with higher meat intake were associated with elevated levels of ApoA1 and CRP, while those specific to processed red meat were associated with increased insulin, HbA1c, and Lp(a) levels. In an independent sample of PEOM and EpiHealth, several meat-associated metabolites were also associated with subclinical markers of CVD and incident CVD, respectively. For example, the metabolite ectoin, which is associated with higher white meat intake, was associated with elevated PWV, while metabolites positively associated with processed red meat were associated with VO_2 max. Our findings on meat-associated metabolites align with those from

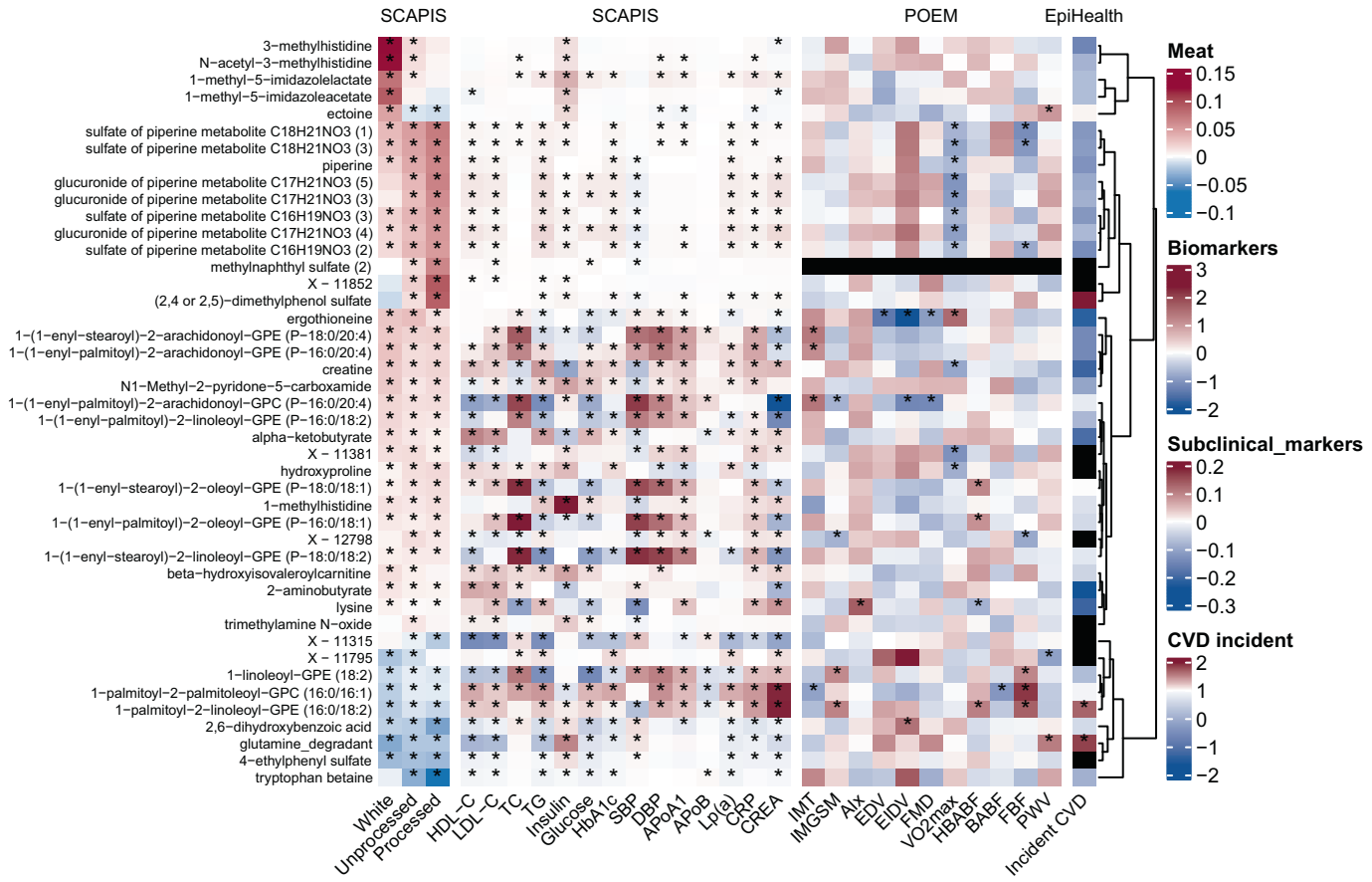

Fig. 4. Heatmap illustrating the associations of the top 20 of white, unprocessed red meat and processed red meat -associated metabolites with cardiometabolic biomarkers, subclinical CVD markers and incident CVD. The 'SCAPIS' column includes the meat types and cardiometabolic biomarkers. The 'POEM' column includes the subclinical CVD markers, and the 'EpiHealth' column includes incident CVD. Cardiometabolic biomarkers and subclinical CVD markers were scaled to the same units prior to statistical analysis. **Abbreviations**: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HbA1c, Hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; Lp(a), lipoprotein (a); CRP, C-reactive protein; CREA, creatinine; IMT, intima-media thickness of the carotid artery; IMGSM, intima-media grayscale median; AIx, fasting augmentation index; EDV, endothelium dependent vasodilation; EIDV, endothelium dependent vasodilation; FMD, flow-mediated vasodilation; VO_2max, maximal oxygen consumption; HBABF, hyperemia brachial artery blood flow; BABF, resting brachial artery blood flow; FBF, resting forearm blood flow; PWV, pulse wave velocity; CVD, cardiovascular diseases. Asterisk (*) indicates statistically significant associations (false discovery rate < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

previously reported in a review article [32]. We observed similar associations between white meat and th 3-methylhistidine and carnitine. Red meat intake, including both unprocessed and processed types, was associated with carnitine and carnosine. However, we were not able to replicate the association between white meat and acetyl-carnitine (C2). In our study, acetyl-carnitine was inversely associated with white meat intake, whereas it was positively associated in the previous study [32]. Our study also revealed that several plasma metabolites were associated with white, unprocessed red, and processed red meat, which can be used for further validation and to expand upon previous studies in this area [15,17–18,33–36].

Among the top 20 meat-associated metabolites, white meat intake was positively associated with 1-methyl-5-imidazolelactate, a product of histidine metabolism formed specifically through the methylation and subsequent decarboxylation of histidine derivatives [37]. This observation is likely due to the high protein content of white meat, which contains derivatives related to histidine metabolism [38,39]. Previous studies reported chicken/poultry intake to be associated with 3-methylhistidine, which is also derived from histidine metabolism [40,41]. The biological significance of 1-methyl-5-imidazoleacetate is less well characterized, and more research is needed to fully elucidate its role. Further, we found that the metabolite 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4) was positively associated with both unprocessed and processed red meat. Our finding is consistent with the prior meat-metabolomic study in the EPIC-Norfolk cohort, which reported that red meat intake was positively associated with 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4) [42]. However, red meat was not further subdivided into unprocessed and processed in that study.

Our study suggests that white meat and unprocessed red meat intake were negatively associated with glutamine degradant indicating a potential role of meat intake in maintaining glutamine reserves. This finding indirectly suggests that vegeterians may have lower levels of specific amino acids, including glutamine precursors, even though they can synthesize glutamine from amino acids present in plant-based foods [43]. Additionally, our study shows that processed red meat was associated with lower plasma levels of 2,6-dihydroxybenzoic acid, though further research is needed to determine the role of this metabolite in human biology.

In our study, we found that white meat-associated metabolites mainly consist of amino acids and lipids. It is plausible that white meat contains generally higher levels of amino acids, such as glutamine compared to red meat [44]. The xenobiotics, such as the sulfate and

glucuronide metabolites of piperine, were associated with both unprocessed and processed red meat. This association could be due to residual confounding or factors specific to meat processing, such as the type of additives and preservatives used in processed red meat. Wedekind et al. reported that pepper alkaloids were associated with the intake of processed meat in one of the European EPIC cohorts [45].

In the present study, the plasmalogen metabolic pathway was positively enriched for metabolites associated with all three meat types, indicating that these meat types were associated with an increase in metabolites involved in plasmalogen metabolism. Plasmalogens are vital for lipid raft and cholesterol-rich membrane stability, crucial for cellular signaling where meat, such as beef, pork, and chicken, is a primary dietary source [46].

Moreover, our enrichment analysis revealed that the observed enrichment of white meat-associated metabolites and branched-chain amino acid metabolic pathways, such as histidine metabolism and leucine, isoleucine, and valine metabolism, is likely due to the direct intake of these metabolites from meat, rather than resulting from metabolic upregulation of these endogenous pathways. This limitation should be considered when interpreting the results, as distinguishing between exogenous and endogenous sources of these metabolites can be challenging and requires further investigation. Our findings suggest that processed red meat metabolites were positively enriched for benzoate and medium chain acyl carnitine metabolism pathways [34]. This suggest that processed red meat may contain foreign chemical compounds that increase the risk of inflammation and metabolic abnormalities [7,34].

We found that unprocessed red meat intake, but not processed red meat, was associated with TMAO, a metabolite formed by gut microbes. Similar to our findings, unprocessed red meat, but not processed red meat, was correlated with TMAO levels in a previous study [3]. However, the literature on meat intake and TMAO is inconsistent. Previous studies have shown a positive association between red meat consumption and TMAO levels [3,47]. Moreover, TMAO, has often been associated with fish consumption [39,48]. These inconsistencies may arise from the overall influence of dietary patterns on the relationship between meat intake and TMAO levels, along with significant variations in meat consumption across different populations, study designs, and dietary habits. Furthermore, the finding that the association of plasma TMAO with higher levels of HDL-C, LDL-C, and glucose may indicate the implications of unprocessed red meat associated TMAO for metabolic health and disease, though causality cannot be inferred in our study. However, the lack of association between plasma TMAO levels and subclinical CVD markers and incident CVD, despite the positive association between elevated plasma levels of TMAO and CVD in previous findings, is noteworthy [3,49]. We speculate that the inconsistent findings may be attributed to the small sample size used in the POEM and EpiHealth cohorts.

Our study revealed specific patterns of meat-associated metabolites and their associations with cardiometabolic biomarkers, subclinical CVD markers, and incident CVD. We found that metabolites positively associated with all three meat types, such as 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4), were associated with higher levels of ApoA1, CRP, and IMT, indicating a potential risk of CVD. Similarly, metabolites negatively associated with all three meat types, such as 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1) were associated with higher insulin levels. This association might result from complex metabolic or lifestyle factors, such as changes in lipid metabolism, affecting insulin sensitivity independently of dietary carbohydrate intake [50],which was relatively lower in the low meat intake group in our study. Moreover, metabolites from processed red meat, including sulfate and piperine, were associated with higher levels of insulin, HbA1c, Lp(a), and lower values of VO_2 max, indicating impaired insulin sensitivity and reduced cardiovascular function with higher intake.

In the present study, the finding that 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2) and a glutamine degradant, which were inversely associated with all three meat types were associated with an increased risk of incident CVD. Lind et al reported that palmitoyl-oleoyl-GPE (16:0/18:1), another fatty acids metabolite integral to lipid metabolism, was associated with incident CVD in the EpiHealth cohort [51]. Our findings indicate that 3-methylhistidine, which was associated with white meat but not with processed red meat, shows an inverse association with CVD. Conversely, dimethylphenol sulfate, which was associated with processed red meat but not white meat, was associated with increased CVD risk. Additionally, ectoine, a metabolite associated with white meat but not processed red meat, was associated with subclinical CVD markers such as a higher value of PWV; however, it was not associated with CVD risk. Although the results for meat associated metabolites and CVD risk may not be significant due to the limited number of cases in EpiHealth, these metabolites might serve as mediators of the effect of meat intake on CVD, warranting further investigation.

This study identified plasma metabolites associated with meat intake and provided insights into the biological mechanisms linking meat consumption to CVD risk. For example, processed red meat-associated metabolites, associated with higher levels of fasting insulin, HbA1c, and Lp(a), as well as their negative correlation with VO_2 max, may contribute to increased cardiovascular risk and reduced fitness. However, the relationship between meat consumption and human metabolism is intricate [52]. Although we adjusted for potential confounders, it is not possible to fully rule out all potential measured and unmeasured confounding effects. Furthermore, the complexity of human metabolism, including individual metabolic variations, dietary habits, and environmental influences, complicates the understanding of the associations between meat intake and plasma metabolites [52,53].

Our study has several strengths. First, a large-scale epidemiological sample enhances the robustness of the data and provides a comprehensive understanding of the associations between meat intake and metabolites. Second, participants were recruited using personal identification numbers, enabling randomized recruitment from the Swedish population register. Third, study participants were well-characterized with detailed phenotypic information, allowing for the control of potential confounders and sensitivity analyses.

However, several limitations need to be acknowledged. First, this is a cross-sectional study, and establishing a causal link between meat intake and plasma metabolites in SCAPIS would benefit from longitudinal study in the future. Second, participants are mainly from Sweden, aged 50–64, which affects generalizability and leaving the need to replicate our findings in different populations across other age groups. Third, the subjective nature of FFQ is imprecise and can be affected by systematic and random errors, such as underreporting of meat intake. Fourth, the relationships between meat intake and plasma metabolites may still be influenced by unmeasured confounders, such as overall dietary patterns, including vegan or plant-based diets, and environmental or psychological factors. Fifth, even though a large number of metabolites were analyzed in our study, there may be metabolites that are associated with meat intake that were not included in the assay. Sixth, missing value replacement for metabolites may introduce bias, as with any imputation technique. Seventh, the small sample size in the POEM cohort and limited number of CVD cases in EpiHealth cohort ($n = 107$) may limit our power to detect associations between meat-associated metabolites and subclinical CVD markers, as well as between metabolites and incident CVD.

5. Conclusion

Our large-scale population-based study identified hundreds of plasma metabolites associated with self-reported white meat, unprocessed red meat, and processed red meat intake. Across all meat types, positively meat-associated metabolites were also associated with cardiometabolic biomarkers such as higher plasma levels of ApoA1, CRP, and markers of subclinical CVD such as IMT. Processed red meat-associated metabolites were further associated with worse glycemic

measures and reduced cardiovascular function. Two metabolites, 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2) and a glutamine degradant, inversely associated with all three meat types were also associated with a higher risk of incident CVD. Our findings highlight specific plasma metabolites that may play a role in the association between meat intake and CVD measures, offering potential insights into the underlying mechanisms. However, further studies are needed to establish causality.

CRediT authorship contribution statement

Getachew Arage: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Conceptualization. **Koen F. Dekkers:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Conceptualization. **Luka Marko Rašo:** Writing – review & editing, Writing – original draft. **Ulf Hammar:** Writing – original draft, Visualization, Validation, Formal analysis. **Ulrika Ericson:** Writing – review & editing, Writing – original draft, Conceptualization. **Susanna C. Larsson:** Writing – review & editing, Writing – original draft. **Hanna Engel:** Writing – review & editing, Writing – original draft, Conceptualization. **Gabriel Baldanzi:** Writing – review & editing, Writing – original draft. **Kamalita Pertiwi:** Writing – review & editing, Writing – original draft. **Sergi Sayols-Baixeras:** Writing – review & editing, Writing – original draft. **Rikard Landberg:** Writing – review & editing, Writing – original draft. **Johan Sundström:** Writing – review & editing, Writing – original draft. **J. Gustav Smith:** Writing – review & editing, Writing – original draft. **Gunnar Engström:** Writing – review & editing, Writing – original draft. **Johan Ärnlöv:** Writing – review & editing, Writing – original draft. **Marju Orho-Melander:** Writing – review & editing, Writing – original draft. **Lars Lind:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Tove Fall:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization. **Shafqat Ahmad:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Author contributions

SA, TF, MOM, SCL, JA, and GE obtained the funding. SA, GA, KFD, UH, HE, and TF designed the study and developed the concept. KFD and TF performed quality control of metabolomics. GA carried out the statistical analyses with contributions from SA and UH. GA, LMR, KFD and SA visualized the results. TF, SA, and MOM coordinated the study. GA, SA, KFD, and LMR wrote the main manuscript. All authors contributed to the critical interpretation of the results, reviewed and revised the paper for important intellectual content, and approved the final manuscript.

Declaration of competing interest

JÄ has served on advisory boards for Astella, AstraZeneca, and Boehringer Ingelheim, and has received lecturing fees from AstraZeneca and Novartis, all of which are unrelated to the present work. JS reports direct or indirect stock ownership in companies (Anagram kommunikation AB, Sence Research AB, Symptoms Europe AB, MinForskning AB) providing services to companies and authorities in the health sector including Amgen, AstraZeneca, Bayer, Boehringer, Eli Lilly, Gilead, GSK, Göteborg University, Itrim, Ipsen, Janssen, Karolinska Institutet, LIF, Linköping University, Novo Nordisk, Parexel, Pfizer, Region Stockholm, Region Uppsala, Sanofi, STRAMA, Takeda, TLV, Uppsala University, Vifor Pharma, WeMind. The remaining authors declare no competing interests. Moreover, the manuscript was previously posted as a preprint on medRxiv (DOI: https://www.medrxiv.org/content/10.1101/2024.10.21.24315788v1).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2025.156188.

Data availability

Access to pseudonymized SCAPIS phenotype data requires ethical approval from the Swedish Ethical Review Board and authorization from the SCAPIS Data Access Board (https://www.scapis.org/data-access). The metabolomics analysis was conducted at Metabolon in Texas, USA, which submitted the spectral data from the initial analytical stage (MS1) for 125 anonymized SCAPIS-Uppsala samples to MetaboLights under accession number MTBLS407. However, MS/MS spectral data are not made available to the research community by Metabolon. Access to individual-level data from EpiHealth and POEM is also restricted due to the presence of sensitive personal information. Permission from the Swedish Ethical Review Authority (https://etikprovningsmyndigheten.se) is required to access these data.

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