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Meat intake in relation to composition and function of gut microbiota



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SUMMARY

Objective: Meat intake is suggested to affect gut microbiome composition and the risk of chronic diseases. We aimed to identify meat-associated gut microbiome features and their association with host factors.

Design: Gut microbiota species were profiled by deep shotgun metagenomics sequencing in 9669 individuals. Intake of white meat, unprocessed red meat, and processed red meat was assessed using a food frequency questionnaire. The associations of meat intake with alpha-diversity and relative abundance of gut microbiota species were tested using linear regression models with adjustment for dietary fiber intake, body mass index, and other potential confounders. Meat-associated species were further assessed for association with enrichment of microbial gene function, meat-associated plasma metabolites, and clinical biomarkers.

Results: Higher intake of processed red meat was associated with reduced alpha microbial diversity. White meat, unprocessed, and processed red meat intakes were associated with 36, 14, and 322 microbiota species, respectively. Species associated with processed red meat were enriched for bacterial pathways like amino acid degradation, while those negatively linked were enriched for pathways like homoacetogenesis. Furthermore, species positively associated with processed red meat were to a large extent associated with reduced trimethylamine N-oxide and glutamine levels but increased creatine and carnitine metabolites, fasting insulin and glucose, C-reactive protein, apolipoprotein A1, and triglyceride levels and higher blood pressure.

Conclusion: This largest to date population-based study on meat and gut microbiota suggests that meat intake, particularly processed red meat, may modify the gut microbiota composition, functional capacity, and health-related biomarkers.

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1. Introduction

Evidence based on observational studies suggests that a high intake of red meat increases the risk of major chronic diseases, such as cardiometabolic diseases [1–6] and colorectal cancer [7,8], as well as mortality [9–12]. The association between meat intake and health outcomes depends on type of meat, with an adverse impact observed primarily for processed red meat, that is, meat that has been processed through salting, curing, smoking or other processes to enhance flavor or improve preservation.

The influence of meat intake on human health may be mediated, at least in part, by the gut microbiota [13]. The gut microbiota has been recognized to have an important role in human physiology, including performing important metabolic functions such as biosynthesis of vitamins, breakdown of indigestible compounds, and interacting with the host through production of beneficial or detrimental metabolites [14,15]. Multiple disease states have been associated with a decrease in gut microbiota diversity which is a hallmark of dysbiosis, so, understanding the microbiota dysbiosis through meat intake could serve to maintain health or treat disease.

Murine studies have revealed that extracted meat proteins effects on the gut microbiota and related physiological pathways [16,17]. A few short (1–4 weeks) intervention studies in healthy adults based on small sample sizes (10–45 participants) have examined the impact of increased intake of different types of meat (e.g., white meat, fried meat, or red meat) on microbial profiles [18–21]. These studies showed changes in the abundance of certain microbial taxa, such as the gram-negative *Dorea* genus [22,23], *Bifidobacterium longum* [20], and *Bifidobacteria* [19]. However, these studies suffered from limited taxonomic resolution (e.g., 16 S rRNA sequencing) of the gut microbiome.

The objective of this study was to extensively explore the relationships between intake of various types of meat (i.e., white meat, unprocessed red meat, and processed red meat) and specific gut microbiota species and their association with host factors, including plasma metabolites and clinical biomarkers. This investigation was conducted using high-resolution metagenomic sequencing within the population-based Swedish CARDioPulmonary BiImage Study (SCAPIS).

2. Methods

2.1. Study population

SCAPIS (<http://www.scapis.org/>) is a nationwide epidemiological cohort comprising 30,154 men and women aged 50–65 years who were invited based on a random extract from the population register from six regions across Sweden between 2013 and 2018 [24]. The current study is based on data from the Uppsala and Malmö arms of SCAPIS. In the Uppsala arm (SCAPIS-Uppsala), diet and fecal shotgun metagenomics data were available for 4775 participants (51 % women). In the Malmö arm (SCAPIS-Malmö), diet and fecal shotgun metagenomics data were available for 4903 participants (53 % women). SCAPIS and the current analyses were approved by the Swedish Ethical Review Authority (Dnr 2010-228-31 M, Dnr 2018-315 B, amendment 2020-06597, and Dnr 2023-07352-01).

2.2. Assessment of dietary intake and covariates

Dietary intake was assessed through a validated food frequency questionnaire, called MiniMeal-Q, which included 75 to 126 food items [25–28]. Participants reported their average consumption of each food item over the past few months. White meat items included chicken or other poultry dishes, including fried, cooked,

deep fried or in pot or casserole. Red meat was categorized as unprocessed red meat (e.g., pork, beef, lamb, and dishes containing minced meat) and processed red meat, which involved additional preservation steps such as smoking, salting, curing, or the addition of chemical preservatives like nitrite or nitrate salts (e.g., sausages, salami, ham, and black pudding). Meat consumption in grams per day was created using reported information on portion size and frequency of consumption. Intake of total energy and dietary fiber was estimated in a similar manner and based on energy and fiber content in all food items reported in the food frequency questionnaire.

Self-reported information on country of birth, educational level, and smoking habits were assessed through validated questionnaires, while information on sex and age were extracted from the Swedish population register. Height and weight were measured at the visit to the test center and body mass index (BMI) was calculated by dividing weight (kg) by height (m²). Brachial arterial blood pressure, including systolic (SBP) and diastolic (DBP) measurements, was automatically measured in both arms. Blood pressure readings were taken before the administration of beta-stimulants (for spirometry) or beta-blockers (before computed tomography scan evaluation). The mean of two blood pressure measures was used.

2.3. Fecal metagenomic sequencing

Fecal metagenomic sequencing in SCAPIS-Uppsala and SCAPIS-Malmö has previously been described in detail [29–31]. At the first visit to the study site, participants were instructed how to collect fecal samples at home in the provided kits and to bring them to the study center during their second visit. After the second visit, fecal samples were kept at –20 °C and within 7 days shipped into the central biobank and kept at –80 °C. DNA extraction, shotgun metagenomic sequencing and taxonomic annotation was performed at Clinical Microbiomics A/S (Copenhagen, Denmark). DNA extraction was performed using NucleoSpin® 96 Soil kits (Macherey–Nagel; Germany) and DNA sequencing was performed using an Illumina Novaseq 6000 system (Illumina, USA). On average, 26.3 million read pairs (standard deviation [SD] ±6.9 millions) were generated for SCAPIS-Uppsala and for 25.3 million read pairs (SD ± 3.8 millions) for SCAPIS-Malmö. DNA sequencing reads that passed quality control were assembled to MEGAHIT (v. 1.1.1) [32] and mapped using BWA-MEM (v. 0.7.16a) [33] to a new gene catalog. The new gene catalog was created based on the samples from SCAPIS-Uppsala, SCAPIS-Malmö, and another study sequenced at the same time and 3486 publicly available samples. Species abundance was estimated using the co-abundant microbial genes that passed the quality control, as described previously [30]. In brief, each species contains a set of 100 specific genes which were selected based on optimal and accurate profiling. A species was being considered as detected, if read pairs were mapped to at least three out of 100 signature genes of that species. Gene counts were normalized to read length and conferred to a species count table. Species counts were transformed into relative abundances. On average, 355 species per individual were identified in SCAPIS-Uppsala and 287 species per individual in SCAPIS-Malmö. Species present in less than 100 of the participants were removed and a total of 1985 identified species were included in the analysis.

2.4. Metabolomics and clinical biomarkers measures

Fasting plasma samples were stored at –80 °C and the metabolomics analyses were performed by Metabolon Inc (Durham, USA), as described previously [29]. Four parallel processes were used for metabolites identification which include, a) reverse phase ultra-

performance liquid chromatography coupled with tandem mass spectroscopy (UPLC-MS/MS) using negative-ion mode electrospray ionization, b) hydrophilic interaction chromatography/UPLC-MS/MS, and two additional reverse phase -UPLC-MS/MS resolutions with positive ion mode electrospray ionization. Metabolon softwares were used for the metabolite's peak identification and identification, and quality control procedures. Metabolites measurement that failed to reach detection threshold were imputed for the minimum value of that metabolite. Annotation of the metabolites were performed based on the internal standards for known compounds and unknown compounds were annotated based on the charge-to-mass ratio, chromatography data and retention time.

Clinical biomarkers including insulin was measured in fasting blood samples using Alinity I reagent kit (Abbott). Venous blood samples were used to measure plasma glucose through hexokinase method (Cobas Roche). For SCAPIS-Malmö, fasting blood samples were used to measure glycated hemoglobin (HbA1c) through turbidimetric method while in SCAPIS-Uppsala HbA1c was measured using capillary electrophoretic system. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using standard techniques [24]. Low-density lipoprotein cholesterol (LDL-C) was calculated through Friedwalds formula. Apolipoprotein A1 (ApoA 1), apolipoprotein B (ApoB), and lipoprotein(a) (Lpa) were measured using fasting EDTA-plasma samples through Alinity C (Abbott). Creatinine was measured through fasting blood plasma samples using enzymatic colorimetric method (Cobas Roche for SCAPIS-Malmö and Architect Abbott for SCAPIS-Uppsala). High-sensitive C-reactive protein (hsCRP) was measured in fasting blood plasma samples through immunoturbidimetric method (Cobas Roache for SCAPIS-Malmö and Architect, Abbott for SCAPIS-Uppsala).

2.5. Statistical analysis

All analyses were performed using Stata (version 18.0) and R software (version 4.1.1 (<https://cran.r-project.org/>)). Gut microbiota alpha and beta diversity were calculated using vegan package in R [30]. For alpha diversity, the Shannon index was calculated for each participant, and the beta diversity was calculated using Bray–Curtis dissimilarity index. Associations between meat variables and beta diversity index were tested using distance-based ANOVA. Distance-based ANOVA identifies meat variables associated with beta diversity and measures the percentage of microbial variation explained by the meat variables.

Microbiota species relative abundances were rank-based inverse normal transformed. Associations between meat variables and relative abundance of gut microbiome species and alpha diversity were tested using multiple linear regression models. We employed three models: a) basic model that adjusted for age, sex, DNA extraction plate, and total energy intake; b) partial model that included the same covariates as in the basic model plus dietary fiber intake, fruit and vegetable intake, alcohol intake, physical activity, smoking, and education; and c) fully adjusted model that included the same covariates as in the partial model plus BMI. We applied false discovery rate (FDR) correction at 5 % to account for multiple testing. All reported estimates from the linear regression analyses were scaled per 20 g per day increment in meat intake, corresponding to about 1 SD increase in white and processed red meat and 0.5 SD increase in unprocessed red meat.

The functional potential of the gut microbiota was characterized by the abundance of the genes within the manually curated get metabolic modules (GMM, version 1.07) [34]. To investigate whether certain gut metabolic modules (GMMs) were over-

represented in the meat-species associations, we performed an enrichment analysis using fgsea v1.18.0 R package [35]. Results were corrected for multiple testing at 5 % FDR correction for both positive and negative associations.

Previous research has found that intakes of white meat, red meat, and/or processed meat are robustly associated with plasma levels of 3-methylhistidine, acetyl-carnitine (C2), and creatinine, and possibly associated with carnitine, carnosine, creatine, glutamine, and trimethylamine N-oxide (TMAO) [36]. We extracted the data for the above listed metabolites from metabolomics data in SCAPIS-Uppsala and SCAPIS-Malmö and examined the association of meat-associated species with these metabolites. Metabolite measures were log-transformed, as described previously [29]. We also analyzed the association of meat-associated species with biomarkers of cardiometabolic health, including glycemic traits (fasting glucose, insulin, and HbA1c), lipids (HDL-C, LDL-C, TC, TG), lipoproteins (ApoA 1, ApoB, and Lpa), kidney function (creatinine), inflammation (high-sensitive CRP), and blood pressure (SBP and DBP). TG and CRP were not normally distributed and were log-transformed. Analyses of species-metabolite and species-biomarker associations were adjusted for age, sex, total energy intake, meat (respective meat type for which we identified an association with species) intake, dietary fiber intake, fruit and vegetable intake, alcohol intake, physical activity, smoking, and education.

3. Results

3.1. Participant characteristics

Daily consumption of white meat, unprocessed, and processed red meat was similar in SCAPIS-Uppsala ($n = 4775$) and SCAPIS-Malmö ($n = 4894$) (Table 1). White meat intake was positively correlated with intake of both unprocessed red meat ($r = 0.34$, p -value < 0.0001) and processed red meat ($r = 0.13$, p -value < 0.0001). There was also a positive correlation between unprocessed red meat and processed red meat intake ($r = 0.39$, p -value < 0.0001).

3.2. Processed red meat intake associates with reduced gut microbiome alpha diversity

To compare the patterns of microbial community structure, we investigated the associations of meat intake with the alpha diversity (estimated using Shannon index) of gut microbiota in the combined sample ($n = 9669$) of the SCAPIS-Uppsala and the SCAPIS-Malmö cohorts (Table S1). White meat intake was not associated with Shannon index in any model. Unprocessed red meat intake was associated (negatively) with Shannon index in the basic model only. Processed red meat intake was negatively associated with Shannon index in the basic and partial model, but not in the fully adjusted model further adjusted for BMI.

3.3. Meat intake in association with beta diversity

White meat and unprocessed red meat intake explained a statistically significant but very small proportion (ranging from 0.01 % to 0.07 % depending on model) of the variance in beta diversity, a measure of overall gut microbial composition across samples (Table S2). Processed red meat intake explained 2.1 % (p -value = 3.3×10^{-182}) in the basic but the estimates are statically non-significant in the partial, and fully adjusted models, respectively.

Table 1
Background characteristics of the study participants.

	SCAPIS-MALMÖ (N = 4894)	SCAPIS-UPPSALA (N = 4775)	Total (N = 9669)
Age at first visit, years	57.45 (4.30)	57.74 (4.39)	57.59 (4.35)
BMI, kg/m ²	27.30 (4.61)	27.02 (4.37)	27.16 (4.50)
Fasting glucose, mmol/L	5.91 (1.32)	5.91 (1.10)	5.91 (1.20)
Fasting insulin, mIE/L	7.56 (6.87)	7.70 (14.66)	7.63 (11.42)
HbA1c, mmol/mol	37.24 (7.28)	36.09 (6.00)	36.67 (6.71)
HDL-C, mmol/L	1.67 (0.53)	1.47 (0.39)	1.57 (0.48)
LDL-C, mmol/L	3.60 (0.96)	3.55 (0.94)	3.57 (0.95)
Total cholesterol, mmol/L	5.43 (1.04)	5.70 (1.07)	5.56 (1.06)
Triglycerides, mmol/L	1.30 (0.87)	1.29 (0.74)	1.29 (0.81)
ApoA 1, g/L	1.61 (0.31)	1.61 (0.30)	1.61 (0.30)
ApoB, g/L	1.00 (0.24)	1.01 (0.24)	1.01 (0.24)
Lpa, g/L	0.23 (0.28)	0.23 (0.27)	0.23 (0.27)
Creatinine, μmol/L	77.33 (14.94)	75.44 (17.85)	76.39 (16.47)
C-reactive protein, mg/L	2.38 (4.22)	2.20 (3.86)	2.29 (4.05)
Systolic blood pressure, mmHg	122.44 (16.41)	125.10 (15.93)	123.76 (16.23)
Diastolic blood pressure, mmHg	74.69 (9.63)	76.95 (9.80)	75.81 (9.78)
Total energy intake, kJ/day	7273.83 (3197.30)	7231.61 (2822.01)	7252.98 (3017.72)
Fibre intake, g/day	19.97 (11.46)	20.37 (10.71)	20.17 (11.10)
Whole grain total, g/day	38.98 (43.35)	44.38 (44.40)	41.65 (43.95)
Alcohol intake, g/day	6.88 (6.84)	6.94 (6.20)	6.91 (6.53)
White meat intake, g/day	23.08 (19.58)	22.64 (17.33)	22.86 (18.50)
Unprocessed red meat intake, g/day	47.76 (35.93)	50.61 (33.79)	49.17 (34.92)
Processed red meat intake, g/day	22.39 (22.01)	21.82 (19.72)	22.11 (20.91)
Gender			
Males	2288 (46.8 %)	2311 (48.4 %)	4599 (47.6 %)
Females	2606 (53.2 %)	2464 (51.6 %)	5070 (52.4 %)
Education			
Not completed elementary school	51 (1.1 %)	22 (0.5 %)	73 (0.8 %)
Elementary school	481 (10.1 %)	312 (6.9 %)	793 (8.5 %)
High school	2281 (47.8 %)	1889 (41.5 %)	4170 (44.7 %)
University	1956 (41.0 %)	2329 (51.2 %)	4285 (46.0 %)
Physical activity			
Never	1480 (31.8 %)	1210 (26.9 %)	2690 (29.4 %)
Not regularly	1065 (22.9 %)	956 (21.2 %)	2021 (22.1 %)
1–2 times/week	860 (18.5 %)	965 (21.4 %)	1825 (19.9 %)
2–3 times/week	695 (14.9 %)	831 (18.4 %)	1526 (16.7 %)
>3 times/week	555 (11.9 %)	544 (12.1 %)	1099 (12.0 %)
Smoking			
Not regular smokers	4234 (89.2 %)	4285 (95.2 %)	8519 (92.1 %)
Regular smokers	512 (10.8 %)	214 (4.8 %)	726 (7.9 %)

For continuous variables the values are presented as mean (SD) while for frequency variables the values are presented as numbers (percentage). Abbreviations: ApoA 1, apolipoprotein A1; ApoB, Apolipoprotein B; BMI, body mass index; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lpa, lipoprotein(a).

3.4. Meat intake in association with the relative abundance of gut microbiota species

White meat intake was associated with the relative abundance of 109, 69, and 36 species in the basic, partial, and fully adjusted regression models (Tables S3, S4, and S5; Fig. 1). Among the 36 associations identified in the full model, white meat intake was positively associated with 19 species and negatively associated with 17 species. The strongest positive association was observed for *Streptococcus australis* ($\beta = 0.059$ per 20 g/day increase of white meat intake, q -value = 7.6×10^{-05}), while the strongest negative association was observed for *B. longum* subsp. *longum* ($\beta = -0.061$, q -value = 1.7×10^{-04}).

Unprocessed red meat intake was associated with 325, 31, and 14 species in the basic, partial, and fully adjusted regression models, respectively (Tables S6, S7, and S8; Fig. 1). The species with the strongest positive and negative associations with unprocessed red meat intake were *Amedibacillus dolichus* ($\beta = 0.020$ per 20 g/day increase of unprocessed red meat intake, q -value = 7.1×10^{-04}) and *Streptococcus mutans* ($\beta = -0.027$, q -value = 4.3×10^{-04}), respectively.

Processed red meat intake was associated with 924, 406, and 322 species in the basic, partial, and fully adjusted regression models, respectively (Tables S9, S10, and S11; Fig. 1). The strongest positive association was observed for *Latilactobacillus sakei* subsp. *sakei* ($\beta = 0.060$ per 20 g/day increase of processed red meat intake, q -value = 1.4×10^{-13}), while the strongest negative association was observed for *Clostridium* sp ($\beta = -0.051$, q -value = 2.1×10^{-08}).

3.5. Meat-associated species enriched for the microbial gene functions

We observed no functional pathway enriched for the white meat-associated species (Table S12). Species associated with unprocessed red meat intake were negatively enriched for the pentose phosphate pathway (oxidative phase), trehalose degradation, glycerol degradation I, alanine degradation I, and sucrose degradation II pathways (all q -values <0.05) (Table S13, Fig. S1). Processed meat-associated species were enriched for 32 metabolic pathways, comprising 26 positively associated pathways and 6 negatively associated pathways (Table S14, Fig. S1). Processed meat positively associated species were more enriched for the threonine

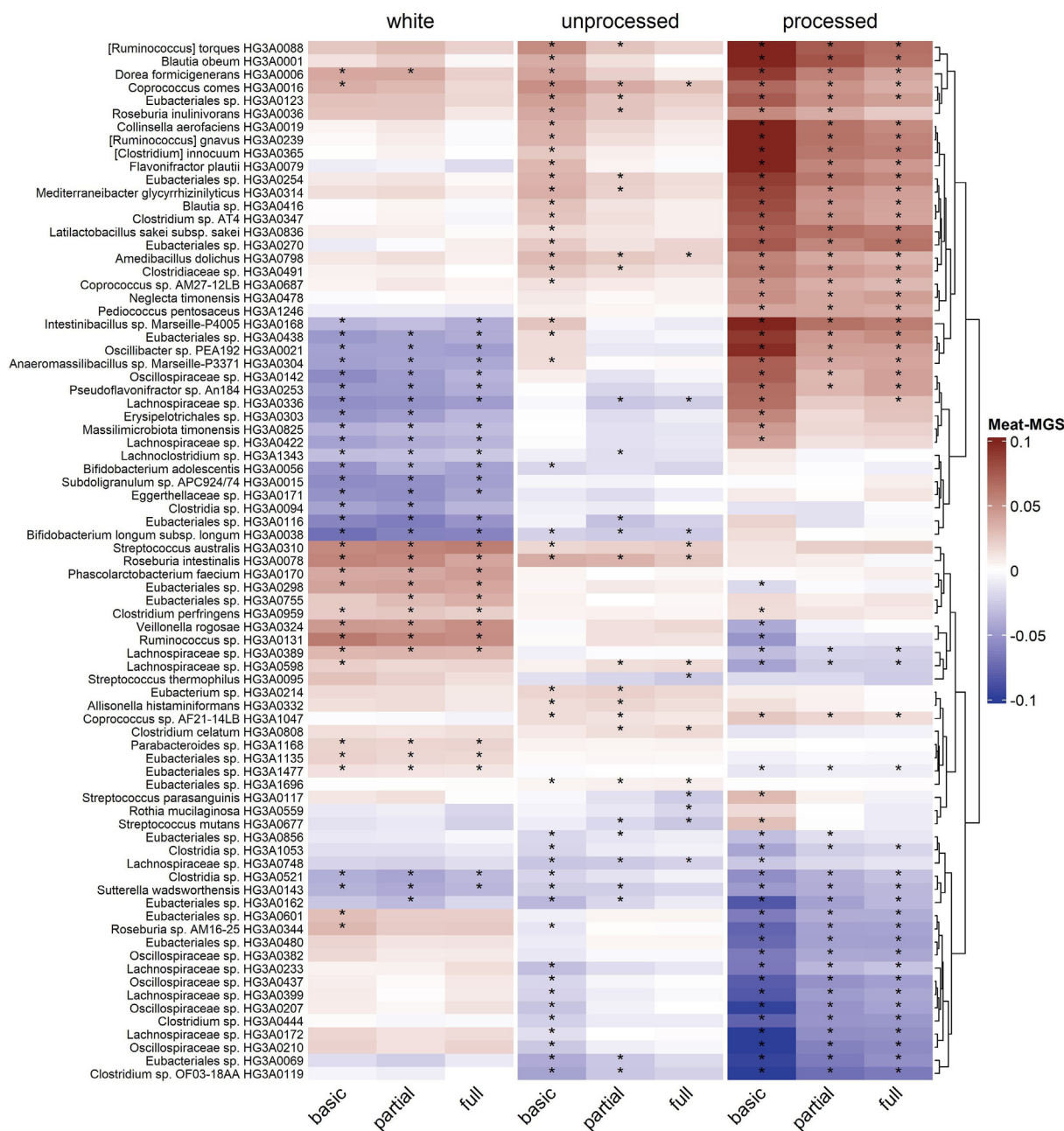


Fig. 1. The top 20 microbiota species associated with white meat intake, all species associated with unprocessed red meat intake, and the top 20 species associated with processed red meat intake. X-axis shows the different model adjustment and y-axis shows the meat-associated species. The basic model was adjusted for age, sex, analytical plate, study site, and total energy intake. Hierarchical clustering was performed based on the association patterns of meat intake with species based on the euclidean distance. The partial model included the same covariates as in the basic model plus dietary fiber intake, fruit and vegetable intake, alcohol intake, physical activity, smoking, and education. The full model included the same covariates as in the partial model plus body mass index. Asterisk (*) denotes statistically significant associations after 5 % False Discovery Rate correction.

degradation I, fructose degradation, pyruvate: formate lyase, and threonine degradation II pathways, while processed meat negatively associated species were enriched for the homoacetogenesis pathway.

3.6. Meat-associated species in association with metabolomic signatures

In the combined analysis of SCAPIS-Uppsala and SCAPIS-Malmö samples (up to n = 8168), we observed that species positively associated with processed red meat were generally

associated with reduced TMAO and glutamine levels but increased creatine, hydroxyproline, acetylcarnitine and carnitine metabolites. Similarly, microbiota species negatively associated with processed red meat were associated with reduced creatine and carnitine metabolites but increased TMAO and 3-methylhistidine levels. Results for the top 20 (selected based on the lowest p-values) each meat type species in association with previously reported meat-associated metabolites (Fig. 2). We observed similar results when all meat-associated microbiota species were analyzed in association with published meat-associated metabolites (Table S15, Fig. S2).

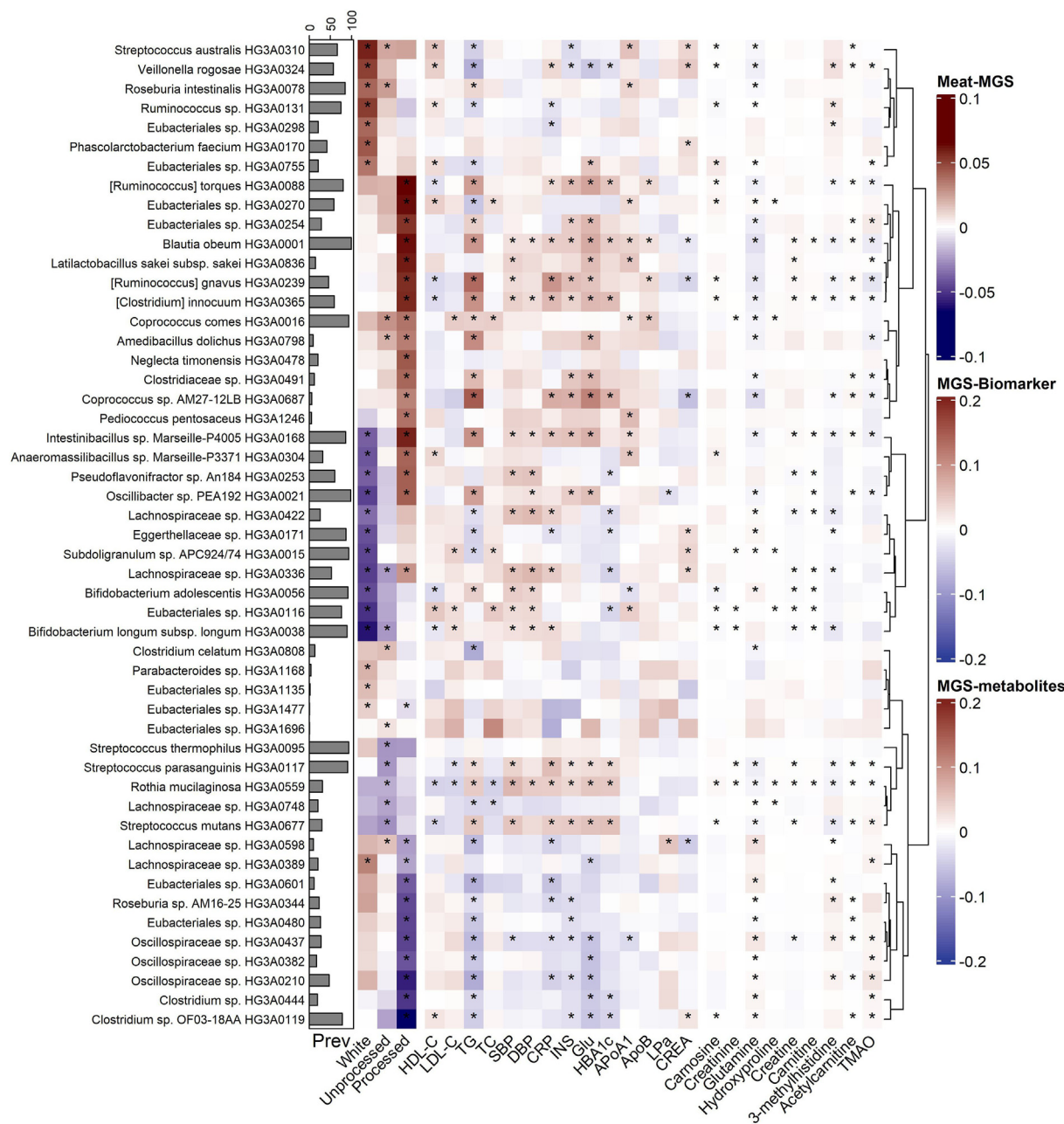


Fig. 2. The top 20 microbiota species associated with white meat intake, all species associated with unprocessed red meat intake, and the top 20 species associated with processed red meat intake in relation to previously reported meat-associated metabolites and clinical biomarkers. Analyses were adjusted for age, sex, total energy intake, meat (respective meat type for which we identified an association with species) intake, dietary fiber intake, fruit and vegetable intake, alcohol intake, physical activity, smoking, and education. Prev, prevalence in percentage; TMAO, trimethylamine N-oxide; ApoA 1, apolipoprotein A1; ApoB, apolipoprotein B; CREA, creatinine; CRP, C-reactive protein; DBP, diastolic blood pressure; Glu, fasting glucose; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; INS, fasting insulin; LDL-C, low-density lipoprotein cholesterol; Lp(a), Lipoprotein A; MGS, microbiota gut species; Prev, prevalence; SBP, systolic blood pressure; TG, triglycerides; TC, total cholesterol. Asterisk (*) denotes statistically significant associations after 5 % False Discovery Rate correction.

3.7. Meat-associated microbiome signatures in association with biomarkers

Next, we analyzed the association of meat-associated microbiota species with clinical biomarkers (Table S16, Fig. 2). When analyzing the association of top 20 species associated with white meat, unprocessed red meat, and processed red meat intake with these biomarkers, we observed that microbial species positively associated with processed red meat were generally positively

associated with increased fasting insulin and glucose levels, TG and ApoA 1 levels, hsCRP levels, and SBP and DBP, but with reduced creatinine levels (Fig. 2). Species that were negatively associated with processed red meat intake were mostly negatively associated with fasting glucose, insulin, HbA1c, and TG levels (Fig. 2, Table S16). We observed similar results when the analyses were adjusted for the other meat types (Table S17). Likewise, similar patterns were observed when all meat-associated species were analyzed in association with biomarkers (Fig. S3).

3.8. Sensitivity analysis

Our findings remained consistent in sensitivity analysis excluding individuals who had used antibiotics within the last year (Tables S18, S19, and S20). Additionally, our results were similar after excluding the most influential observations.

4. Discussion

This hitherto largest study, involving 9669 adults, on meat intake in relation to gut microbiota showed that higher processed red meat intake was associated with a decreased alpha diversity after adjustment for other dietary and lifestyle factors, although the association was attenuated after further adjustment for BMI. Species positively associated with processed red meat were enriched for 26 bacterial metabolic pathways, including amino acid degradation, and species negatively associated with processed red meat were enriched for 6 metabolic pathways, including homoacetogenesis. Moreover, species positively associated with processed red meat were associated with reduced TMAO and glutamine levels but with increased creatine and carnitine levels, and generally also with increased levels of cardiometabolic risk markers (e.g., fasting glucose, insulin, hsCRP, ApoA 1, TG, and SBP and DBP). Intake of white meat and unprocessed red meat was associated with fewer microbiome features compared to processed red meat.

Our findings are consistent with previous studies showing associations between meat intake and gut microbiota composition (summary of previous findings is presented in Table S21). We observed that meat-associated species may influence the functional capacity of the gut microbiome, which is largely driven by the digestion of complex macromolecules [37,38]. Unsurprisingly, we found that most gene enriched pathways are related to degradation of proteins (e.g., tyrosine degradation I). Nevertheless, some gene enriched functional pathways stand out due to their potential role in host health. For example, the pentose phosphate pathway (oxidative phase) is related to cancer cell growth and inflammation [39]. Also, species observed to be positively associated with processed red meat upregulate the propionate production II pathway, and levels of propionate in the bloodstream have been associated with pathological mechanisms behind cardiomyopathy [40].

In regards to abundance of specific species, in our study, all meat types were generally negatively associated with abundance of *Lachnospiraceae* species, which is in line with a previous study where the same direction was reported for processed red meat [41]. Species positively associated with processed red meat intake (e.g., *Ruminococcus torques*, *Coprococcus comes*, and *Blautia obeum*) were associated with higher levels of hydroxyproline, creatine, and carnitine, but with reduced levels of glutamine, 3-methylhistidine, and TMAO. We also observed that some species positively associated with processed red meat intake (e.g., *B. obeum*) were associated with higher levels of cardiometabolic disease biomarkers, such as SBP and DBP, fasting glucose, insulin, TG, CRP, and ApoA 1 but reduced levels of creatinine. A previous study found similar results in participants with prediabetes [42]. *B. obeum* species has previously been associated with increased risk of coronary artery calcium score, a measure of atherosclerosis [30], as well as with higher 24-h SBP [43] in the SCAPIS cohort.

The species negatively associated with white meat intake (e.g., *Eggerthellaceae* sp.) were associated with a lower creatine concentration. Dietary creatine supplementation has been proposed to have high therapeutic potential in vascular health, potentially serving both direct and antioxidant roles by reducing the concentration of low-density and total cholesterol [44]. More visible patterns were observed for TMAO. Diets rich in trimethylamine (TMA)-containing nutrients, including choline, phosphatidylcholine, and

carnitine, can serve as carbon fuel sources for the gut microbiota. The waste product of TMA is carried through the portal circulation to the liver, where it is converted by flavin monooxygenases into TMAO [45]. Species negatively associated with higher white-, unprocessed- and processed red meat intake were associated with increased TMAO levels. Several studies have reported that intake of animal products, such as red meat, is associated with higher circulating levels of TMAO, which has been associated with increased risk of atherosclerosis, thrombosis, and CVD [46–51]. Other species (*Intestinibacillus* sp. *Marseille-P4005*, *Eubacteriales* sp., *Anaeromassilibacillus* sp. *Marseille-P3371*, *Pseudoflavonifractor* sp. *An184* and *Oscillibacter* sp. *PEA192*) that were negatively associated with white meat and positively associated with processed red meat were associated with lower 3-methylhistidine levels. 3-Methylhistidine has gained attraction as a potential biomarker of meat intake [36], specifically for white meat, but is also related to muscle turnover in frailty [52]. We observed that higher intake of white meat and unprocessed red meat was associated with a lower relative abundance of *B. longum* subsp. *longum*. *In vivo* studies have reported that *Bifidobacteria*, which are commonly used as probiotics and accounts for 2–14 % of the gut microbiota in adults, could downregulate TMAO levels [53]. Experimental studies have also shown that increased levels of *Bifidobacteria* were accompanied by reduced levels of TMAO [53,54]. Consistent with these findings, we observed that a higher relative abundance of *B. longum* subsp. *longum* was associated with reduced plasma levels of acetylcarnitine and TMAO.

The reason why intake of processed red meat, as compared to white meat and unprocessed red meat, has been more consistently associated with increased risk of cardiometabolic diseases [1–6] and colorectal cancer [8], remains unclear but could possibly be related to a more pronounced alteration of the gut microbiota by processed red meat, as suggested by our study. Compared to unprocessed meat, processed meat products like sausages, salami, bacon, and liver pâté generally have a higher content of saturated fat, which may negatively affect microbiota richness and diversity [55]. Furthermore, nitrite and nitrate salts that are usually added to processed meat products may affect the composition and function of the gut microbiota, which is involved in metabolizing dietary nitrite and nitrate into reactive nitrogen compounds [56]. A study in mice demonstrated that a high dose of dietary nitrite caused upregulations of several microbiota taxa (e.g., *Alistipes*, *Prevotella*, and *Ruminococcus*) and that the gut microbiota mediated the toxicity of dietary nitrite [57].

The current study is strengthened by the large population-based study sample which improves the generalizability of the findings. Study participants were well characterized, including comprehensive assessment of validated dietary intake, collection of stool samples, and detailed data on potential confounding factors. Additionally, the gut microbiota was characterized by deep shotgun sequencing, which has higher resolution than 16 S rRNA sequencing [58].

There are some limitations of the study that should be noted. First, even though the sample size is large, only participants aged 50 to 65 with a mostly Swedish ancestry were included. Replications are needed to infer the generalizability in other populations. Information on meat intake and confounding factors were obtained through self-reports which can introduce measurement errors. In addition, only relative abundances of species are used entailing issues of compositional data [59]. The microbial composition varies along the longitudinal axis of the intestines, as the human lower gastrointestinal tract contains a variety of specific microbial habitats along the small intestine, cecum, and large intestine. Fecal samples in the present study were primarily derived from the distal colon and less so from other sites of the gastrointestinal tract. Thus,

our findings may not be generalizable to microbiota composition in other parts of the gastrointestinal tract. Lastly, because of the observational and cross-sectional design of this study, the direction and causality of the associations between meat intake, gut microbiota, metabolites, cardiometabolic markers are difficult to disentangle. The Mendelian randomization method, which can improve causal inference in observational data, could not be applied in the present study due to the lack of solid and non-pleiotropic instrumental variables for meat intake. For example, genetic variants associated with meat intake explain a tiny proportion of the variance in meat intake (resulting in weak instrument bias and low statistical power in the analysis) and are also associated with the intake of other foods and mediating traits (e.g., educational attainment and health-related factors) that act on reporting food intake and not food intake itself [60,61].

5. Conclusion

In conclusion, we have observed significant associations between intake of meat, particularly processed red meat, and gut microbiota composition, functional capacity, metabolites, and biomarkers. Our deeply measured dietary, omics, and biomarker data shed light on various physiological pathways. Specifically, microbiota species positively associated with processed red meat were associated with reduced levels of TMAO and glutamine but increased levels of creatine and carnitine metabolites, and generally higher levels of cardiometabolic risk markers and atherosclerosis. These findings advance current research on meat intake and its relationship with gut microbiota. Future studies investigating whether these gut microbiota species might serve as an intermediate link between meat intake and cardiometabolic diseases will be crucial.

Ethics approval and consent to participate

SCAPIS and the current analyses were approved by the Swedish Ethical Review Authority (Dnr 2010-228-31M, Dnr 2018-315B, amendment 2020-06597, and Dnr 2023-07352-01).

Availability of data and materials

De-identified metagenomic sequencing data for SCAPIS samples is available in the European Nucleotide Archive under accession number PRJEB51353 (<https://www.ebi.ac.uk/ena/browser/view/prjeb51353>). The phenotype dataset supporting the findings of this research article was provided by the SCAPIS Data Access Board and is not publicly available due to confidentiality. This data can be shared upon reasonable request to the corresponding author, but only after obtaining permission from the Swedish Ethical Review Authority (<https://etikprovningmyndigheten.se>) and the SCAPIS Data Access Board (<https://www.scapis.org/data-access/>).

Authors' contributions

SCL, GE, JÄ, MO-M, TF and SA obtained the funding for the study. SCL, TF, MO-M, SA planned and designed the study. SA carried out the statistical analyses and GA produced the figures with contribution from UE, KFD, and UH. SCL, LMR, UH, SA wrote the main manuscript. All authors contributed with the critical interpretation of the results, reviewing, editing and approved the manuscript.

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Conflict of interest

HBN and JBH are employees of Clinical Microbiomics. JS reports direct or indirect stock ownership in companies (Anagram kommunikation AB, Sence Research AB, Symptoms Europe AB, Min-Forskning 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, Parxel, Pfizer, Region Stockholm, Region Uppsala, Sanofi, STRAMA, Takeda, TLV, Uppsala University, Vifor Pharma, WeMindstock ownership in Anagram kommunikation AB and Symptoms Europe AB, unrelated to the present study. 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. The remaining authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2024.12.034>.

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