A dietary pattern promoting gut sulfur metabolism is associated with increased mortality and altered circulating metabolites in low-income American adults

Kui Deng,[a] Lei Wang,[a] Sang Minh Nguyen,[a] Martha J. Shrubsole,[a,c] Qiuyin Cai,[a] Loren Lipworth,[a] Deepak K. Gupta,[b] Wei Zheng,[a] Xiao-Ou Shu,[a] and Danxia Yu[a,*]

[a]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Centre, Vanderbilt University Medical Centre, Nashville, TN, USA
[b]Division of Cardiovascular Medicine, Department of Medicine, Vanderbilt Translational and Clinical Cardiovascular Research Centre, Vanderbilt University Medical Centre, Nashville, TN, USA
[c]International Epidemiology Field Station, Vanderbilt University Medical Center, Nashville, TN, USA

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Summary

Background Excessive hydrogen sulfide in the gut, generated by sulfur-metabolising bacteria from foods, has been linked to intestinal inflammation and human diseases. We aim to investigate the interplay between diet and sulphur-metabolising bacteria in relation to mortality and circulating metabolites in understudied populations.

Methods In the Southern Community Cohort Study (SCCS), a prospective cohort of primarily low-income American adults, habitual diets were assessed using a food frequency questionnaire at baseline (2002–2009). A sulfur microbial diet score (SMDS) was developed among 514 Black/African American participants by linking habitual dietary intakes with the abundance of sulfur-metabolising bacteria profiled by faecal shotgun metagenomics. The SMDS was then constructed among all eligible SCCS participants (50,114 Black/African American and 23,923 non-Hispanic White adults), and its associations with mortality outcomes were examined by Cox proportional hazards model and Fine–Grey subdistribution hazard model. The association between SMDS and 1110 circulating metabolites was examined by linear regression among 1688 SCCS participants with untargeted metabolomic profiling of baseline plasma samples.

Findings Over an average 13.9-year follow-up, SMDS was associated with increased all-cause mortality (HR [95% CI] for the highest vs. lowest quartiles: 1.21 [1.15–1.27]) and cardiovascular disease (1.18 [1.08–1.29]), cancer (1.13 [1.02–1.25]), and gastrointestinal cancer-specific (1.22 [1.00–1.49]) mortality among Black/African American participants (all P-trend<0.05). The associations were largely consistent across participant subgroups. Similar results were observed among non-Hispanic White participants. The SMDS was associated with 112 circulating metabolites, which mediated 36.15% of the SMDS-mortality association (P = 0.002).

Interpretation A dietary pattern promoting sulfur-metabolising gut bacteria may contribute to increased total and disease mortality in low-income American adults.

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Keywords: Dietary patterns; Gut microbiota; Metabolomics; Prospective studies; Racial minorities; Low-income population

Introduction

Diet is among the most important modifiable factors for human health and longevity.[1,2] Several dietary patterns, such as the Healthy Eating Index (HEI) per the *Dietary Guidelines for Americans*,[3] Mediterranean diet,[4] and Dietary Approaches to Stop Hypertension (DASH),[5] have been recommended over the past ~30 years to improve human health. Increasing evidence implicates

*Corresponding author. 2525 West End Avenue, Nashville, TN 37203, USA.
E-mail address: danxia.yu@vanderbilt.edu (D. Yu).

Research in context

Evidence before this study

Excessive hydrogen sulfide in the human gut, generated by sulfur-metabolising bacteria from foods, may lead to intestinal inflammation and human diseases. A dietary pattern related to those bacteria (Sulfur Microbial Diet) has been associated with risks of colorectal cancer, obesity, and nonalcoholic fatty liver disease among primarily middle-class White adults.

Added value of this study

Leveraging a population-based cohort of primarily low-income American adults and faecal metagenomics and blood metabolomics data, we constructed a Sulfur Microbial Diet Score (SMDS) and found it associated with significantly increased all-cause and cardiovascular disease, cancer, and gastrointestinal cancer-specific mortality. We further identified circulating metabolites that mediated a significant portion of the SMDS-mortality association.

Implications of all the available evidence

Our study suggests that a dietary pattern linked to sulfur-metabolising gut bacteria may have adverse health effects, including among understudied low-income American adults.

gut microbiota as mediators of the association between diet and human health.[6–8] However, few dietary patterns to date have considered the gut microbial profile.[7,9,10]

Many human gut bacteria (e.g., *Bilophila* and *Desulfovibrio*) can metabolise dietary sulfur-containing compounds, which could lead to excessive hydrogen sulfide (H_2S) production in the gastrointestinal (GI) tract. Excess H_2S may cause epithelial DNA damage,[11] epithelial cell apoptosis,[12] intestinal mucosa damage,[13] and intestinal inflammation,[14] leading to GI diseases, such as inflammatory bowel disease[15,16] and colorectal cancer (CRC).[17] Different dietary components have varying effects on gut microbial sulfur metabolism.[18] For example, sulfur-containing amino acids may generate H_2S,[19] while dietary fibres, resistant starch, and fructans may suppress H_2S production. Given the impact of diet on microbial sulfur metabolism and H_2S production, recent studies developed a novel dietary pattern according to the abundance of sulfur-metabolising gut bacteria. The Sulfur Microbial Diet Score (SMDS) has been associated with increased risk of CRC in the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study II (NHSII), cohorts of predominantly middle-class, non-Hispanic White adults in the United States (US).[9,10,20] The SMDS has also been linked to risks of obesity and nonalcoholic fatty liver disease (NAFLD) in the UK Biobank.[21,22] To our knowledge, no study has evaluated SMDS and its association with health outcomes and blood metabolites among low-income or Black/African American individuals. Given the considerable heterogeneity in dietary habits and gut microbial profiles across populations from different geographical locations and ethnic origins,[23,24] it is important to develop a specific SMDS and evaluate its associations with health outcomes among low-income individuals and Black/African American individuals who face disproportionally high disease burden.

Therefore, we constructed a de novo SMDS among Black/African American participants with faecal shotgun metagenome data in the Southern Community Cohort Study (SCCS), a prospective cohort of predominantly low-SES American adults living in the southeastern US. We then examined the associations of SMDS with all-cause and cardiovascular disease (CVD) and cancer-specific mortality among 50,114 Black/African American and 23,923 non-Hispanic White participants in the SCCS with an average follow-up of >13 years. Additionally, given the central role of metabolism in linking diet and human diseases,[25,26] we applied global metabolomics and identified circulating metabolites associated with SMDS to help understand the potential underlying metabolic effect of the sulfur microbial diet on human health.

Methods

Study population

Detailed information on the SCCS was described in a previous paper[27] and can be found on the cohort website (https://www.southerncommunitystudy.org). Briefly, the SCCS enrolled ~85,000 participants aged 40–79 years, predominantly low-income, uninsured/underinsured adults (~65% were self-reported Black/African American and >80% had annual household income <$25,000) from 12 southeastern US states between 2002 and 2009.[27] Participants were surveyed at baseline using validated structured questionnaires to obtain information on sociodemographics, lifestyle, disease history, and medication use.[27] They were followed up through surveys and national and state linkages to disease and death registries. Venous blood samples were collected at the same time as the baseline survey and transported at 4 °C to the Molecular Epidemiology Laboratory of Vanderbilt University Medical Center and stored at −80 °C for long-term use.

We developed the SMDS in a subset of Black/African American participants with diet and faecal shotgun metagenomics data (N = 514). We evaluated the associations between SMDS and mortality outcomes among all SCCS participants with diet and mortality data, including 50,114 Black/African American and 23,923 non-Hispanic White American adults. The

metabolites analysis with SMDS was performed in a subset of SCCS participants with baseline plasma metabolomics data (N = 1688). The study design is shown in Fig. 1.

Diet assessment

Habitual diet in the past 12 months was assessed at baseline through an 89-item food frequency questionnaire (FFQ), which captured the primary sources of energy and nutrient intakes for adults living in the southeastern US.[28,29] The FFQ in SCCS was validated against 24-h dietary recalls and demonstrated a high level of agreement (kappa = 0.82–0.96 for macronutrients and 0.73–0.95 for micronutrients).[28] The intakes of food items were adjusted for total energy intake, standardised to 2000 kcal/day, and categorised into 33 groups based on the similarity of nutrient profiles or culinary usage per United States Department of Agriculture (USDA) food composition databases and similar to food groups used in previous studies on SMDS.[9,10,30–32] Intakes of food groups were standardised into zero-mean and unit-variance. The HEI and DASH score were calculated and described in our earlier publications.[33,34]

Mortality ascertainment

Vital status, date, and underlying cause of death were obtained via linkages to the National Death Index and Social Security Administration vital status service for epidemiologic research through December 31, 2020. Deaths due to CVD (I00–I78), cancer (C00–C97), and GI cancer (C18–C21, colorectal cancer; C22, liver cancer; C25, pancreatic cancer; and C16, stomach cancer) were ascertained by International Classification of Diseases (ICD)-10 codes.

Stool sample collection

In the follow-up survey between 2018 and 2020, all SCCS participants who remained available to contact were asked if they were willing to provide a stool sample, and those who agreed were mailed a kit for collecting and returning the stool sample. Approximately 8000 SCCS participants collected a stool sample using the faecal occult blood test following a standard protocol.[35] Participants were also asked to complete a short survey, which included the date/time of sample collection, whether they had undergone a colonoscopy, sigmoidoscopy, or other procedure requiring bowel preparation in the past two months, history of diarrhoea, and any antibiotic use in the past two months. Stool samples and the completed survey were then shipped with prepared postage to the Molecular Epidemiology Laboratory of the Vanderbilt Epidemiology Center and stored at –80 °C until further use.

Shotgun metagenomic sequencing

Among Black/African participants who donated stool samples, 600 were randomly selected for shotgun metagenomic sequencing. DNA extraction was performed using the DNeasy PowerSoil Kit (Qiagen, Carlsbad, CA, USA, catalogue #47016) following the manufacturer's protocol. Libraries for whole-genome shotgun metagenomic sequencing were constructed from the DNA samples using a short-insert library protocol and sequenced at paired-end 150 bp on the DNBSEQ platform (BGI Americas, Cambridge, MA, USA). DNA extraction, library preparation, and sequencing were done in a single batch.[36] An average of 17.5 million raw reads (range: 12.2–18.9 million) were obtained. Trimmomatic (version 0.39) was used to trim low-quality reads.[37] Bowtie2 (version 2.3.0) was used to remove the reads aligned to the human genome (*H. sapiens*, UCSC hg19).[38] Based on the method to develop SMDS (9), taxonomic profiling was performed using MetaPhlAn2 (version 2.6.02) with default settings, which used a library of clade-specific markers to provide pan-microbial quantification at the species level.[39]

For the present analysis, we excluded those with low-quality sequencing data (n = 6), missing dietary information (n = 42), prevalent inflammatory bowel

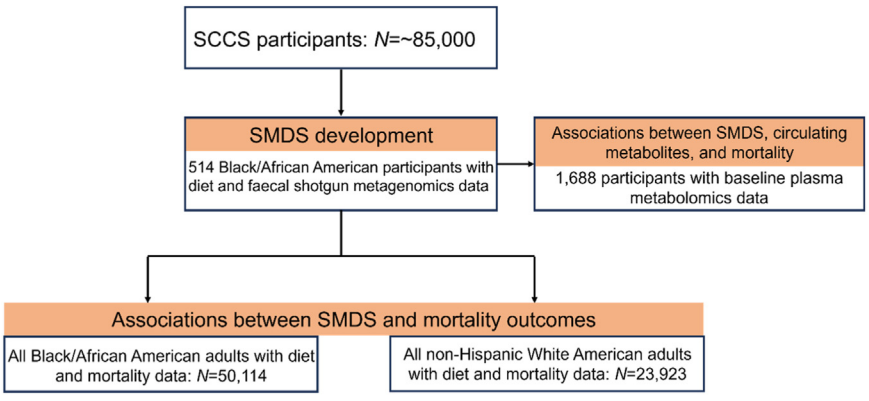

Fig. 1: **The flowchart of study design.**

disease (n = 2), colonoscopy, sigmoidoscopy, or other procedure requiring bowel preparation (n = 30), diarrhoea (n = 4), or antibiotics use (n = 2) in the past 2 months before stool collection. Finally, 514 participants were included to construct the SMDS. We extracted the abundance of 43 sulfur-metabolising species, which were identified in a previous study based on experimental evidence and the presence of sulfur-modifying enzymes in the pangenomes.[9] Microbial species present with relative abundance ≥0.01% in <10% of participants were excluded, leaving 38 species in our analysis. The relative abundances of microbial species were log-transformed (zero values were replaced with 1e-05) and standardised to zero-mean and unit-variance.

Plasma metabolites profiling

Plasma metabolites profiling was conducted in a subset of SCCS participants (n = 1688) who had complete dietary data, provided plasma samples at baseline, and were selected in nested case–control studies of incident coronary heart disease (CHD) (n = 1023)[40] and incident prostate cancer (n = 665). For both studies, the included participants had no history of any cancer. For the CHD study, participants also had 1) no history of CHD, stroke, heart failure, or end-stage renal disease at baseline; 2) data on fasting time and time between sample collection and lab processing; 3) no use of antibiotics and no cold/flu in last seven days before blood sample collection (to avoid the influence of recent infection); 4) eligibility for Centres for Medicare & Medicaid Services and had ≥2 claims after SCCS enrolment (to facilitate CHD case identification). Ultra-high-performance liquid chromatography coupled with tandem mass spectrometry was performed by Metabolon (Morrisville, NC),[40,41] which detected ∼1500 metabolites in plasma samples. After excluding metabolites with a missing rate >20%, 1110 metabolites were used in the analysis. Other missing metabolites were imputed by half the minimal values of non-missing data.[42–44] Metabolite levels were log-transformed and standardised to zero-mean and unit-variance before analysis.

Statistics

SMDS derivation among Black/African American participants with microbiome data

Based on 38 sulfur-metabolising microbial species and 33 food groups among 514 Black/African American participants with microbiome data, we used sparse canonical correlation analysis (SCCA) to identify a latent vector with a subset of food groups that were maximally correlated with another latent vector with a subset of sulfur-metabolising bacteria, with optimal parameters determined by 100 permutation tests. The SCCA selected 23 food groups and 28 microbial species to construct the sulfur microbial dietary pattern. We calculated SMDS by summing the standardised intakes of selected food groups weighted by their canonical weights. A higher SMDS represented greater alignment with the sulfur microbial dietary pattern. The SCCA was implemented using R package PMA (version 1.2–2),[45] which has demonstrated good performance for microbiome data analysis.[10,46] Partial Spearman correlations between selected food groups and microbial species were calculated, adjusting for age, sex, and total energy intake.

SMDS with total and cause-specific mortality in large SCCS cohort

Among all eligible SCCS participants, i.e., 50,114 Black/African American adults and 23,923 non-Hispanic White adults, SMDS was calculated using the weights of selected food groups obtained from the subset of participants with gut microbiome data. We also calculated the SMDS developed by Wang et al. (SMDS_Wang) in the HPFS and NHSII.[10] Pearson correlations of our SMDS with SMDS_Wang, HEI-2010, and DASH score were calculated. We categorised SMDS into four groups based on sex-specific quartiles and examined the associations of SMDS quantiles with all-cause mortality using Cox proportional hazards regression, with the lowest quartile as the reference group, based on two models with sequential adjustment: model 1) included age (continuous), sex (male or female), education (<high school, high school, or college), annual household income (<$15,000, $15,000–$24,999, or ≥$25,000), smoking status (current, former, or never), total physical activity (metabolic equivalent of task [MET] hours/week, continuous), and total energy intake (continuous); model 2) additionally included body mass index (BMI) (continuous) and histories (yes or no) of cancer, CVD, diabetes, hypertension, and hypercholesterolaemia. We further adjusted for HEI-2010 or DASH score based on model 2 to see if SMDS could provide additional information beyond the currently recommended dietary patterns. Missing values of covariates (<2%) were replaced with sex-specific median (for continuous variables) or mode (for categorical variables) values of the non-missing ones. Visual inspection of Schoenfeld residuals against time confirmed no violations of the proportional hazards assumption. We used Fine–Grey subdistribution hazard competing risk regression to examine the associations of SMDS quantiles with CVD, cancer, and GI cancer-specific mortality, with all other causes of death except the one we studied as competing risk events, adjusting for the same covariates mentioned above. P for trend was obtained by treating the median SMDS in each quartile as a continuous variable. We also assessed the associations of sex-specific quantiles of the SMDS_Wang with all-cause and cause-specific mortality.

Sensitivity analyses were performed by excluding 1) participants with a history of CVD or cancer at baseline and 2) participants who died within 5 years to

minimise the chance of reverse causality. Subgroup analyses were conducted by sex, current smoking status, BMI (<30 or ≥30 kg/m^2), diabetes status, hypertension status, and hypercholesterolaemia status, with *P* for interaction obtained from the corresponding interaction term. Our main results were presented separately for Black/African American and non-Hispanic White participants.

SMDS with circulating metabolites

We used linear regression to identify plasma metabolites associated with SMDS (zero-mean and unit-variance), adjusted for age, sex, self-reported race, education, income, smoking status, physical activity, total energy intake, BMI, cancer, CVD, diabetes, hypertension, hypercholesterolaemia, fasting status, and assay batch. The Benjamini–Hochberg method was used to control the false discovery rate (FDR). The normality and homoscedasticity assumptions of the linear regression were checked by the normal quantile plot of the residuals and the scatterplot of residuals compared with predicted values, respectively, and no violations were found. An unweighted metabolite score was constructed by summing the standardised abundance of SMDS-associated metabolites and considering the direction of the association. We then examined the associations of SMDS-related metabolites and metabolite score with all-cause mortality using Cox proportional hazards regression, adjusting for the same covariates. Mediation analysis was performed to evaluate the potential mediation effects of metabolites/metabolite score on the association between SMDS and all-cause mortality, using R package mediation (version 4.5.0).[47] To ensure the interpretability of the SMDS-metabolite-mortality relations, we only included metabolites showing the same direction of associations with SMDS and with all-cause mortality in the mediation analysis.

All statistical analyses were performed in R (version 4.3.2). Two-sided $P < 0.05$ or FDR<0.05 was considered statistically significant.

Ethics

The SCCS was approved by the Institutional Review Boards of the Vanderbilt University Medical Center and Meharry Medical College (#010345), and written informed content was obtained from all participants.

Role of funders

The funders were not involved in any part of the study design, data collection, data analyses, interpretation, or writing of report.

Results

Characteristics of study participants

The main analysis of SMDS and mortality included SCCS participants who self-reported as Black/African American or non-Hispanic White, had complete dietary data, and survived at least one year after enrolment, i.e., a total of 74,037 participants, including 50,114 Black/African American and 23,923 non-Hispanic White adults. The SMDS was constructed among 514 Black/African participants who had complete dietary data at baseline and donated stool samples during follow-up (see Methods). Compared with Black/African American participants included in the SMDS-mortality analysis, those with faecal microbiome data were slightly older (mean age: 53.2 vs. 51.5 years; Table 1), had more women (72% vs. 59.1%), similar BMI (mean: 30.9 vs. 30.6 kg/m^2), and similar follow-up durations (13.8 vs. 13.9 years). Meanwhile, among non-Hispanic White participants included in the SMDS-mortality analysis, the mean age was 54.1 years, 39% were men, mean BMI was 29.9 kg/m^2, and mean follow-up was 13.1 years.

The sulfur microbial diet score

A total of 23 food groups/beverages and 28 sulfur-metabolising microbial species were selected in the SCCA, and these food groups/beverages and their canonical weights were used to construct the SMDS (Fig. 2). The sulfur microbial dietary pattern was characterised by high intakes of animal-source foods, soups, sauces, and gravies, alcoholic beverages, fried potatoes, coffee, refined grains, and high-energy drinks, while low intakes of low-fat or fermented dairy products, vegetables and fruits, whole grains, nuts, tea, and legumes. The SMDS was inversely correlated with HEI-2010 ($r = -0.71$) and DASH score ($r = -0.70$) and positively correlated with ultra-processed food intake (UPF; $r = 0.49$) and the SMDS developed by Wang et al. (hereinafter referred to as SMDS_Wang) in the HPFS and NHSII(46) ($r = 0.61$); all $P < 0.001$ (Pearson correlation). Participants with higher SMDS were younger, had lower education and income levels, were more likely to be current smokers, and had higher total energy intake, but were less likely to have prevalent chronic diseases, suggesting possible dietary changes after disease diagnosis (Table S1).

Associations of SMDS with total and cause-specific mortality

Among 50,114 Black/African American participants, 14,342 died during follow-up, including 4667 from CVD, 3341 from cancer, and 863 from GI cancer. After adjustment for sociodemographic, lifestyle, and health factors, SMDS was significantly associated with all-cause and CVD, cancer, and GI cancer-specific mortality, with hazard ratio [HR] and 95% confidence interval [CI] in the highest vs. lowest quartiles [Q4 vs. Q1]: 1.21 (1.15–1.27), 1.18 (1.08–1.29), 1.13 (1.02–1.25), and 1.22 (1.00–1.49) respectively; all *P*-trend <0.05 (Cox model for all-cause mortality and Fine–Grey model for cause-specific mortality) (Fig. 3). Further adjustment for

	Microbiome dataset (*n* = 514)	Black/African American participants (*n* = 50,114)	Non-Hispanic White participants (*n* = 23,923)
Age, years, mean (SD)	53.2 (7.7)	51.5 (8.5)	54.1 (9.1)
Male, *n* (%)	144 (28.0)	20,481 (40.9)	9330 (39.0)
Female, *n* (%)	370 (72.0)	29,633 (59.1)	14,593 (61.0)
Education, *n* (%)			
<High School	83 (16.1)	15,541 (31.0)	5482 (22.9)
High school	342 (66.5)	29,458 (58.8)	13,728 (57.4)
College	89 (17.3)	5115 (10.2)	4713 (19.7)
Annual household income, *n* (%)			
<$15,000	217 (42.2)	29,789 (59.4)	11,105 (46.4)
$15,000–$24,999	129 (25.1)	11,134 (22.2)	4370 (18.3)
≥$25,000	168 (32.7)	9191 (18.3)	8448 (35.3)
Smoking status, *n* (%)			
Current	137 (26.7)	21,008 (41.9)	8948 (37.4)
Former	133 (25.9)	10,201 (20.4)	6885 (28.8)
Never	244 (47.5)	18,905 (37.7)	8090 (33.8)
Total physical activity, MET-hours/week, mean (SD)	21.8 (17.0)	22.8 (19.0)	21.5 (17.7)
Total energy intake, kcal/day, mean (SD)	2437.5 (1357.9)	2685.3 (1522.4)	2269.7 (1212.8)
Body mass index, kg/m^2, mean (SD)	30.9 (6.8)	30.6 (7.5)	29.9 (7.4)
Cardiovascular disease, *n* (%)	36 (7.0)	5365 (10.7)	3459 (14.5)
Any cancer, *n* (%)	34 (6.6)	2283 (4.6)	3524 (14.7)
Diabetes, *n* (%)	80 (15.6)	11,197 (22.3)	4625 (19.3)
Hypertension, *n* (%)	286 (55.6)	29,015 (57.9)	11,682 (48.8)
Hypercholesterolaemia, *n* (%)	200 (38.9)	15,015 (30.0)	9999 (41.8)
Follow-up duration, years, mean (SD)	13.8 (2.0)	13.9 (4.1)	13.1 (4.0)

MET, metabolic equivalent of task; SD, standard deviation.

Table 1: **Baseline participant characteristics in the Southern Community Cohort Study.**

HEI-2010 or DASH score did not attenuate the associations of SMDS with all-cause mortality (HR [95% CI] for Q4 vs. Q1: 1.13 [1.06–1.20] and 1.19 [1.12–1.27] for HEI and DASH adjustment, respectively), CVD mortality (1.13 [1.01–1.25] and 1.22 [1.10–1.37] respectively), or GI cancer mortality (1.27 [1.00–1.64] and 1.38 [1.08–1.78] respectively), suggesting SMDS may provide additional information beyond the currently recommended dietary patterns. Adjustment for UPF also did not attenuate (even strengthened) the SMDS-mortality association. By comparison, SMDS_Wang was only associated with all-cause mortality, with a smaller HR than our SMDS (1.08 [1.03–1.14] vs. 1.21 [1.15–1.27]). Also, SMDS_Wang was not associated with CVD (HR [95% CI]: 1.05 [0.96–1.15]), cancer (1.05 [0.94–1.16]), or GI cancer-specific mortality (1.07 [0.88–1.31]) among Black/African American participants in our cohort.

The associations of SMDS with all-cause and CVD mortality were similar in sensitivity analyses by excluding individuals with prevalent CVD or cancer at enrolment or excluding those who died within 5 years (Fig. S1). The SMDS-mortality associations were largely consistent across participant subgroups defined by sex, tobacco smoking, and statuses of obesity, diabetes, hypertension, and hypercholesterolaemia (all *P*-interaction >0.05, Cox model for all-cause mortality and Fine–Grey model for cause-specific mortality). See Fig. 4 for all-cause mortality, Fig. S2 for CVD-specific mortality, and Fig. S3 for cancer-specific mortality. A stronger association of SMDS with all-cause mortality was observed in women than in men (HR [95% CI] for Q4 vs. Q1: 1.26 [1.17–1.36] in women and 1.15 [1.07–1.23]; *P*-interaction = 0.018; Fig. 4).

Among 23,923 non-Hispanic White participants (Table S2), higher SMDS was also associated with higher all-cause (HR [95% CI] for Q4 vs. Q1: 1.13 [1.05–1.22]) and CVD-specific mortality (1.16 [1.01–1.33]), but not with cancer-specific mortality (0.96 [0.83–1.12]) (Fig. S4). SMDS_Wang was only associated with all-cause mortality (HR [95% CI] for Q4 vs. Q1: 1.09 [1.02–1.17]), but not with CVD or cancer-specific mortality among White participants in the SCCS.

Associations of SMDS with circulating metabolites

We identified 112 metabolites associated with SMDS (FDR <0.05, linear regression; Table S3), among which 36 showed the same direction of associations with SMDS and with all-cause mortality (n_{death} = 688) (FDR <0.05, Cox regression; Fig. 5). Each of these 36 metabolites mediated the association between SMDS and all-cause mortality (proportion mediated: 3.7–17.7%,

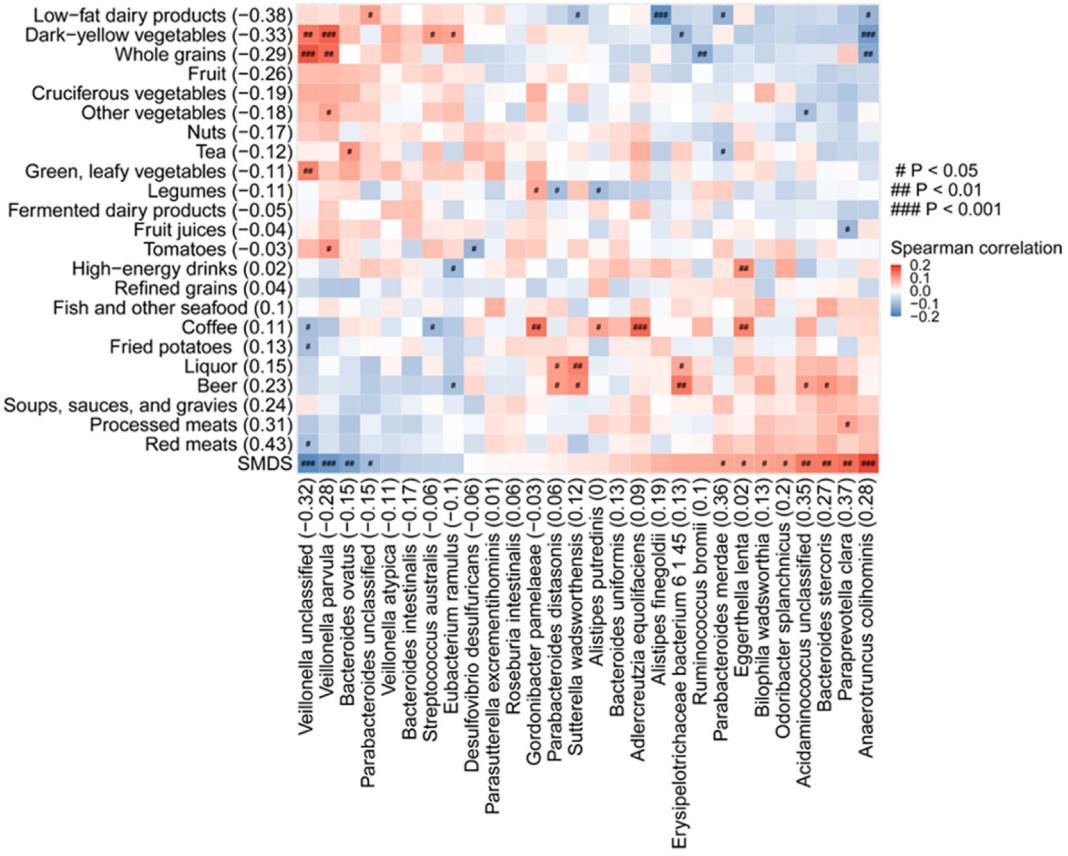

Fig. 2: **Partial Spearman correlations of selected food groups and the constructed SMDS with microbial species among 514 Black/African American participants in the Southern Community Cohort Study**. Correlation analysis was adjusted for age, sex, and total energy intake. The numbers in parenthesis in the column and row are the canonical weights for the food groups and microbial species, respectively, obtained by the sparse canonical correlation analysis. The canonical weights of food groups were used to construct the sulfur microbial diet score. SMDS, sulfur microbial diet score.

FDR<0.05, mediation analysis; Fig. 5). In addition, a metabolite score combining all 36 metabolites mediated 36.2% of the association between SMDS and all-cause mortality (P = 0.002, mediation analysis).

Discussion

In this large cohort of predominantly low-SES American adults in the southeastern US, we identified a dietary pattern related to sulfur-metabolising gut bacteria and found the SMDS associated with significantly increased all-cause and CVD, cancer, and GI cancer-specific mortality, with HRs of 1.13–1.22 across quartiles after adjusting for major mortality risk factors. We further identified circulating metabolites that mediated a large portion of the association between SMDS and increased mortality. Our findings demonstrate the adverse health effect of a diet related to sulfur-metabolising gut bacteria and the potential metabolic profiles underlying this dietary pattern and its adverse effect.

Accumulating evidence has linked the sulfur microbial diet with both GI and non-GI-related diseases, including CRC,[9,10,20] obesity,[21] NAFLD,[22] and ovarian cancer survival.[48] Our study used the same method (SCCA) to construct the SMDS as the study conducted by Wang et al.,[10] while Nguyen et al. used a different method (reduced rank regression).[9] Other studies used the weights obtained from Wang et al. or Nguyen et al.'s study to construct the SMDS.[21,22,48] Our study extends previous studies by constructing a de novo SMDS and examining its associations with all-cause and leading causes of death (CVD and cancer) in a large cohort of predominantly low-SES American adults, who have disproportionately high morbidity and mortality while have been underrepresented in research, particularly gut microbiome research. All these studies, including ours, suggest the detrimental health effect of a dietary pattern promoting sulfur-metabolising gut bacteria. Specifically, our study recommends limiting the intake of red meats, processed meats, alcoholic beverages,

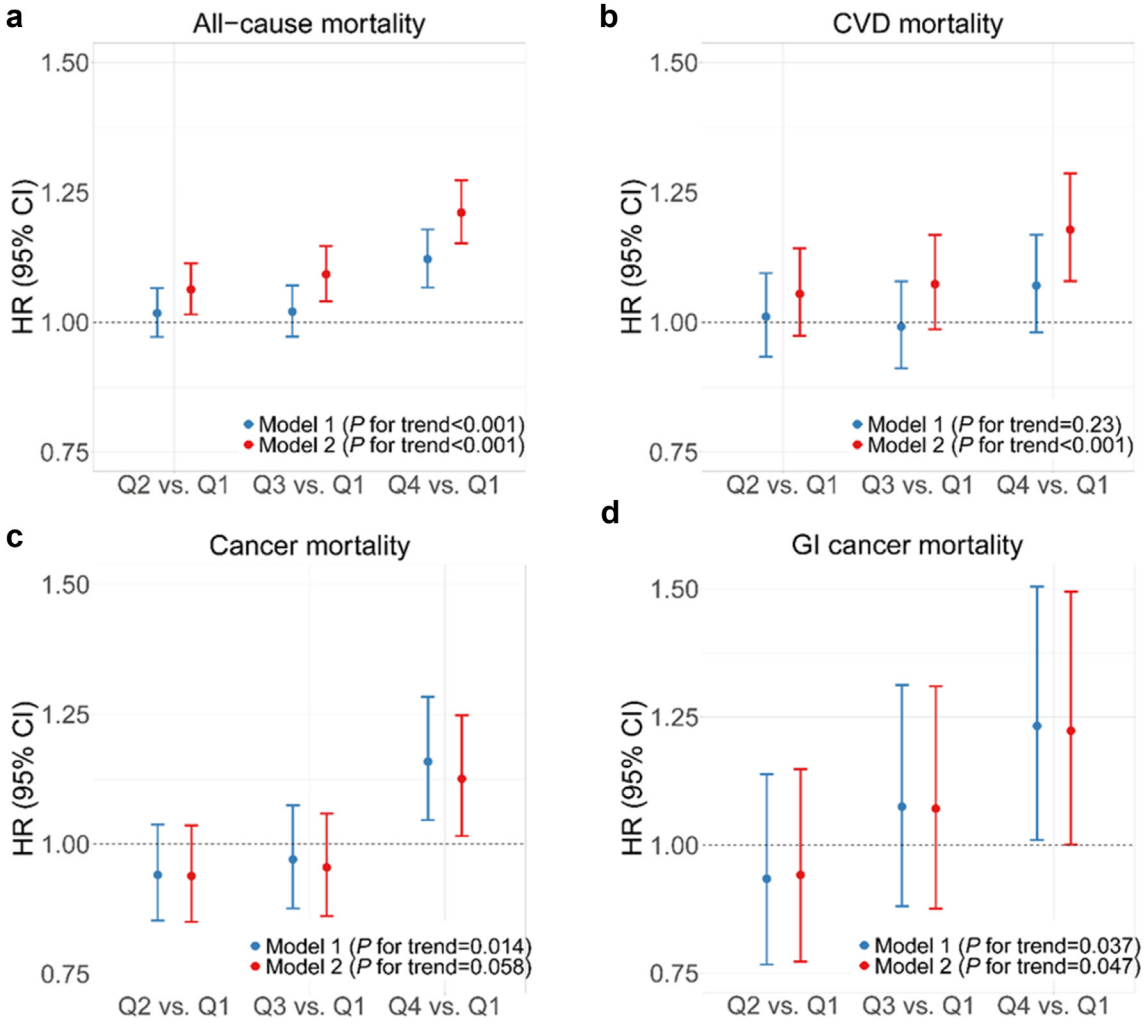

Fig. 3: **Associations of the sulfur microbial diet with all-cause and CVD, cancer, and gastrointestinal cancer-specific mortality among 50,114 Black/African American participants in the Southern Community Cohort Study**. **(a)** Association between the sulfur microbial diet and all-cause mortality. **(b)** Association between the sulfur microbial diet and CVD-specific mortality. **(c)** Association between the sulfur microbial diet and cancer-specific mortality. **(d)** Association between the sulfur microbial diet and gastrointestinal cancer-specific mortality. Cox proportional hazards regression and Fine–Grey subdistribution hazard competing risk regression were used to examine the associations of sulfur microbial diet score with all-cause and cause-specific mortality, respectively. Model 1 included age, sex, education, income, smoking status, physical activity, and total energy intake; model 2 additionally included BMI and histories of cancer, CVD, diabetes, hypertension, and hypercholesterolaemia. *P* value for trend was obtained by treating the median sulfur microbial diet score in each quartile as a continuous variable. Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; CVD, cardiovascular disease; GI, gastrointestinal; HR, hazard ratio; CI, confidence interval.

high-energy drinks, and fried potatoes, and increasing the intake of foods rich in fibre, low-fat dietary products, fermented dairy products, and tea. Given the different mortality rate, dietary habits, and gut microbiome compositions across racial/ethnic and SES groups,[23,24] our findings are important to validate and extend the adverse health effects of a sulfur microbial diet in diverse populations.

Our identified sulfur microbial diet, to some extent, reflected an unhealthy dietary pattern opposite to the current recommendations, as indicated by high negative correlations with HEI and DASH score. Nevertheless, we found the SMDS-mortality association was independent of HEI and DASH score, suggesting our identified sulfur microbial dietary pattern may capture emerging health-related factors, i.e., the gut microbiota and microbiota–host interaction. Compared with HEI and DASH, our SMDS additionally included tea, fermented dairy products, and some alcoholic beverages such as beer and liquor (Fig. 2). Recent population-

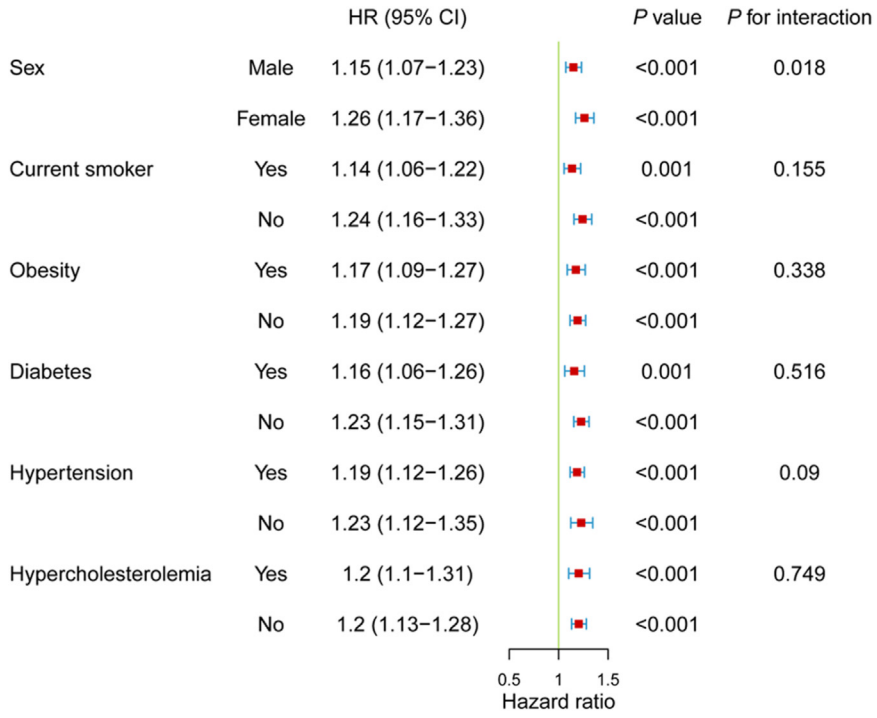

Fig. 4: **Subgroup analyses of the association between the sulfur microbial diet and all-cause mortality among Black/African American participants in the Southern Community Cohort Study**. The associations were examined by Cox proportional hazards regression, adjusted for potential confounders. *P* for interaction was obtained from the corresponding interaction term in the model. HR, hazard ratio; CI, confidence interval.

based studies have shown that habitual tea consumption was inversely associated with chrovnic insomnia-disrupted gut microbiota (e.g., *Ruminococcaceae UCG-002* and *Ruminococcaceae UCG-003*), hypertension-related gut microbiota (e.g., *Coprococcus catus*), and bile acids (e.g., isolithocholic acid, muro cholic acid and nor cholic acid).[49,50] Fermented dairy products (e.g., yogurt, cheese, and fermented milk) have been well known for their beneficial effects on human gut microbiota and host physiology.[51–53] For example, fermented dairy products could increase the abundance of possibly beneficial bacteria, including *Bifidobacterium* genus, *Adlercreutzia equolifaciens*, and *Slackia isoflavoniconvertens*.[51] Conversely, alcohol intake has been linked to impaired gut microbiota, as shown in recent human studies.[36,54] Taken together, the sulfur microbial dietary pattern may capture gut microbiome-related mechanisms beyond the established dietary patterns, while its effects on specific gut microbes and how the interplay between diet and microbiota may affect human health warrant further investigation.

The SMDS generated using microbiome data in our study sample showed stronger associations with mortality outcomes among Black and White SCCS participants than the SMDS_Wang, which was generated in the HPFS and NHSII,[10] although they were correlated (r = 0.61) and shared many components, including food groups with positive weights (e.g., red meats, processed meats, liquor, fried potato, and coffee) and food groups with negative weights (e.g., low-fat dietary products, fruits and vegetables, fruit juice, whole grains, nuts, and legumes). Animal-based foods, e.g., red meat and processed meats, are rich in sulfur-containing amino acids, which could be fermented by gut bacteria to release H_2S.[55,56] H_2S may destroy mucus layers, lead to intestinal inflammation and epithelial damage, and increase gut permeability.[57] In contrast, foods rich in fibre (e.g., fruits and vegetables, whole grains, nuts, and legumes) were inversely correlated with abundances of sulfur-metabolizing bacteria, as shown in Fig. 2. Higher fibre intake is associated with lower H_2S production in humans and animals,[56,58,59] which may be partly due to the increased production of short-chain fatty acids that may lower pH and abundances of sulfur-metabolising bacteria, thereby reducing H_2S.[58,60] Overall, our study supports previous findings that limiting animal-based foods and increasing dietary fibre intake may exert health benefits by reducing sulfur-metabolising bacteria and H_2S production in the gut. Meanwhile, the stronger associations with mortality observed for our de novo SMDS suggested that microbiome-related dietary patterns may vary somewhat

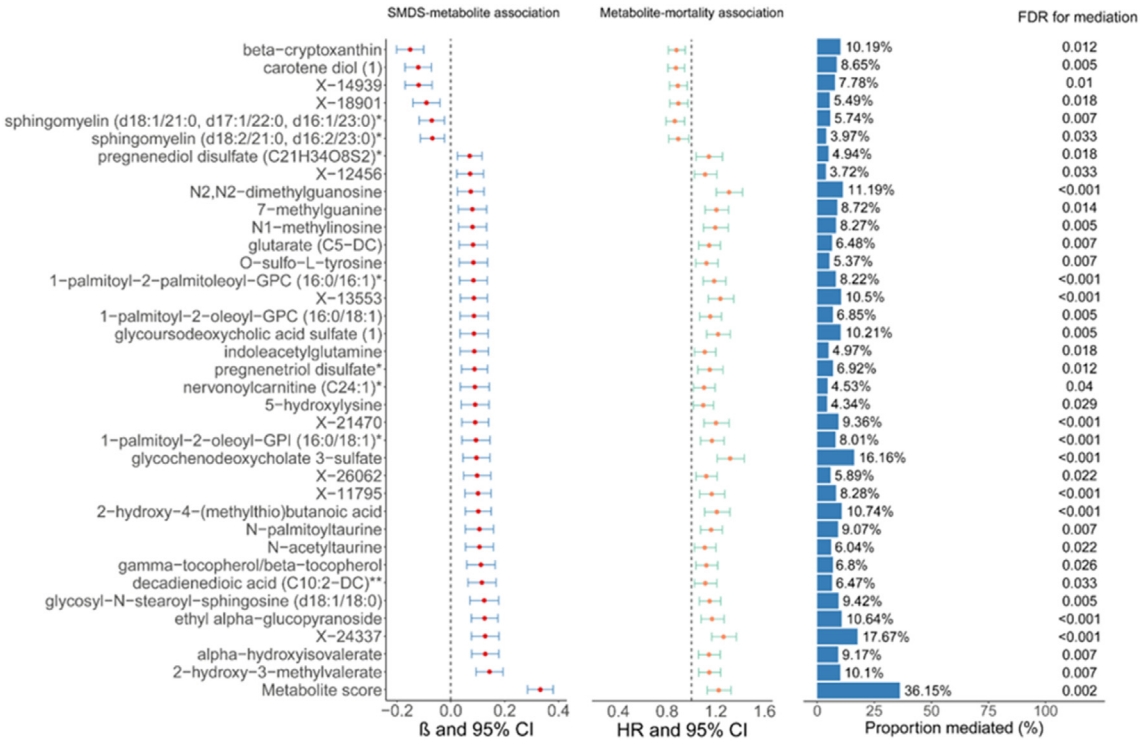

Fig. 5: **The role of circulating metabolites in linking sulfur microbial diet and mortality**. The associations between sulfur microbial diet score and metabolites/metabolite score were estimated by linear regression, adjusted for age, sex, self-reported race, education, income, smoking status, physical activity, total energy intake, BMI, cancer, CVD, diabetes, hypertension, hypercholesterolaemia, fasting status, and assay batch. The associations between metabolites/metabolite score and all-cause mortality were estimated by Cox proportional hazards regression, adjusted for the same covariates as above. Mediation analysis was used to evaluate the potential mediation effects of metabolites/metabolite score on the SMDS-mortality association. FDR was controlled by the Benjamini–Hochberg method. SMDS, sulfur microbial diet score; FDR, false discovery rate; CI, confidence interval.

between populations, highlighting the importance of diverse study participants in evaluating the generalisability and enhancing public health implications of research findings.

In addition, our study identified circulating metabolites correlated to the sulfur microbial diet that may mediate the latter association with mortality. Most of these metabolites are annotated as lipids and amino acids. For example, 2-hydroxy-4-(methylthio)butanoic acid and N-acetyltaurine, both positively associated with SMDS and mortality, belong to the methionine, cysteine, S-adenosylmethionine (SAM) and taurine metabolism pathway, which is an important sulfur metabolism pathway for H_2S production through the metabolisation of methionine contained in meat.[61] Glycochenodeoxycholate 3-sulfate and glycoursodeoxycholic acid sulfate (1), belonging to the bile acid metabolism pathway, mediated large portions of the SMDS-mortality association (>10%). Bile acid metabolism is one of the most important microbial pathways relevant to human health,[62] and our findings support the role of bile acids in linking diet, gut microbial sulfur metabolism, and disease.

The main strengths of our study include a large prospective cohort with a long-term follow-up, a detailed dietary survey, and the availability of gut microbiome and blood metabolites data in subsets of cohort participants for *de novo* constructing the SMDS and evaluating the underlying metabolic profiles. Also, most participants are Black/African American and non-Hispanic White adults with low SES, who are underrepresented in prospective studies and omics studies. We also acknowledge several limitations of the current study. First, the time lag between dietary assessment at baseline and stool collection during cohort follow-up (~14 years) may bias the association between dietary intakes and gut microbiome and make the SMDS more prone to measurement error, as the participants' diets may change over time. Nevertheless, the SMDS performed better than SMDS_Wang, highlighting the importance of considering the dietary background and gut microbial profiles of the study population. Second, although we have adjusted for major confounding factors, residual confounding caused by imperfect adjustment or unadjusted confounders cannot be ruled out, given the observational nature of the study. Unadjusted

confounders might include other prevalent diseases, such as neurological, renal, and gastrointestinal diseases, which may affect dietary choices and mortality outcomes. Third, gut microbiome and blood metabolome data were not measured at the same time point, which prevented us from directly assessing the associations between sulfur-metabolising bacteria and SMDS-related metabolites. Meanwhile, the SMDS was developed from Black/African American participants only. Thus, our findings should be further validated in other cohort studies with matched gut microbiome and blood metabolome data and with racially and geographically diverse populations.

In summary, we identified a sulfur microbial dietary pattern among low-SES Black/African American individuals, characterised by higher intakes of red meats, processed meats, alcoholic beverages, high-energy drinks, and fried potatoes, while lower intakes of foods rich in fibre, low-fat dietary products, fermented dairy products, and tea. This dietary pattern was significantly associated with increased all-cause and CVD and cancer (including GI cancer) mortality among Black/African American and non-Hispanic White participants. Circulating metabolites may mediate the association between sulfur microbial diet and mortality. Our findings suggest the adverse effect of a diet that promotes sulfur-metabolising gut bacteria on health and longevity among understudied populations facing high burden of disease.

Contributors

DY designed the study. KD analysed the data and draughted the manuscript. LW, SMN, MJS, QC, LL, DKG, WZ, and XOS provided critical revision of the manuscript for important intellectual content. All authors contributed to the acquisition of data and interpretation of the results. DY is the guarantor of the work and, as such, has full access to all the data in the study and takes responsibility for its integrity and accuracy. All authors read and approved the final manuscript.

Data sharing statement

The data underlying this article can be obtained through the Southern Community Cohort Study (https://www.southerncommunitystudy.org/) under application 571 and data access request 435 upon reasonable request and approval by the SCCS Data and Biospecimen Use Committee.

Declaration of interests

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105690.

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