Association of the sulfur microbial diet and biological aging: a cross-sectional study of 71,579 participants

Ye Liu [1]*; Dan He [1]*; Yifan Gou [1]; Ruixue Zhou [1]; Chen Liu [1]; Jingni Hui [1]; Meijuan Kang [1]; Bingyi Wang [1]; Panxing Shi [1]; Feng Zhang [1]#

[1]Key Laboratory of Trace Elements and Endemic Diseases of National Health and Family Planning Commission, School of Public Health, Health Science Center, Xi'an Jiaotong University, Xi'an, China

*The two authors contributed equally to this work

#Corresponding author: Feng Zhang, Key Laboratory of Trace Elements and Endemic Diseases, National Health Commission of the People's Republic of China. School of Public Health, Health Science Center, Xi'an Jiaotong University, Email: fzhxjtu@mail.xjtu.edu.cn, Xi'an, P. R. China 710061

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Abstract

The sulfur microbial diet (SMD), a dietary pattern associated with 43 sulfur-metabolizing bacteria, may influence gut microbiota composition and contribute to aging process through gut-produced hydrogen sulfide (H_2S). We aimed to explore the association between SMD and biological age acceleration, using the cross-sectional study included 71,579 individuals from the UK Biobank. The SMD score was calculated by multiplying β-coefficients by corresponding serving sizes and summing them, based on dietary data collected using the Oxford WebQ, a 24-hour dietary assessment tool. Biological age (BA) was assessed using Klemerae-Doubal (KDM) and PhenoAge methods. The difference between BA and chronological age refers to the age acceleration (AgeAccel), termed "KDMAccel" and "PhenoAgeAccel". Generalized linear regression was performed. Mediation analyses were used to investigate underlying mediators including body mass index (BMI) and serum aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio. Following adjustment for multiple variables, a positive association was observed between consuming a dietary pattern with a higher SMD score and both KDMAccel (β_{Q4vsQ1} = 0.35, *95%CI* = 0.27 to 0.44, P<0.001) and PhenoAgeAccel (β_{Q4vsQ1} = 0.32, *95%CI* = 0.23 to 0.41, P<0.001). Each 1-standard deviation increase in SMD score was positively associated with the acceleration of biological age by 7.90% for KDMAccel (P<0.001) and 7.80% for PhenoAgeAccel (P<0.001). BMI and AST/ALT mediated the association. The stratified analysis revealed stronger accelerated aging impacts in males and smokers. Our study indicated a higher SMD score is associated with elevated markers of biological aging, supporting the potential utility of gut microbiota-targeted dietary interventions in attenuating the aging process.

***Keywords*:** Sulfur microbial diet; biological age; Klemerae Doubal method; PhenoAge.

Abbreviation list:

SMD, the sulfur microbial diet; H_2S, hydrogen sulfide; BA, biological age; KDM, Klemerae-Doubal method; AgeAccel, age acceleration; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CA, chronological age; FEV1, forced expiratory volume in one second; SBP, systolic blood pressure; TDI, townsend deprivation index; CHD, coronary heart disease; ISCED, International Standard Classification of Education; SE, standard error; RCS, restricted cubic spline; CI, confidence interval.

1. Introduction

Aging represents an irreversible and intricate process, a primary risk factor for many significant human diseases[1]. As the population ages, health care costs increase[2]. However, individuals with the same chronological age (CA) exhibit variations in the rate of aging and various susceptibilities to many age-related diseases, indicating that CA is not a perfect measurement[3]. Therefore, there is a need for a better understanding of the aging process and identification of the determinants of biological aging.

The dysbiosis of the microbiota related to aging contributes to the reshaping of immune responses during the aging process[4]. It is accompanied by numerous age-related diseases both within and outside the gastrointestinal tract. The modifiable regulation of the gut microbiota suggests it is a potential target for interventions in aging[4]. Meanwhile, nutrition is considered an effective regulatory factor influencing health and aging, and a new discipline has been established: “Nutrigerontology”, which combines insights from biogerontology, nutrition, and medicine to understand the impact of diet and nutrition on the aging process and age-related diseases[5]. Diet is also a key modifiable factor influencing gut microbiota composition[6].

Researchers utilized dietary data from the Nurses' Health Study II (NHSII) to construct a gut microbiota-derived dietary pattern: the sulfur microbial dietary pattern (SMD), which is characterized by a low intake of fruits, vegetables, legumes, whole grains, and nuts, along with a high intake of red meat, processed meat, high-fat dairy products, sugary beverages, and coffee. Among them, processed meat, liquor, and low-calorie drinks were found to be positively associated with the enrichment of sulfur-metabolizing microbes, while the remaining five components showed an inverse association[7]. Sulfur-metabolizing microbiota can convert dietary sulfur into genetically toxic hydrogen sulfide (H_2S), which can cause DNA damage[8] and promote alterations in immune cells associated with inflammation and cancer[9]. The association between SMD and various diseases such as non-alcoholic fatty liver disease[10] and obesity[11] has been studied in the UK Biobank. However, little is known about its association with aging.

"Biological age (BA)"[12] is crucial in understanding aging, providing a comprehensive evaluation of aging across multiple bodily systems[13], and measuring the accumulation of damage over time in individuals[14]. Markers of BA exhibit significant predictive power for mortality, age-related ailments, and declines in bodily function[15]. Various BA methodologies such as the Klemera-Doubal (KDM) and the PhenoAge methods were devised to delineate the heterogeneity of aging based on respiratory, metabolic, renal, immune, and cardiovascular functions[16]. Numerous studies on BA have emerged, including its association with dietary inflammatory index [17], macronutrients[18], and dietary oxidative balance[19]. However, no research has yet explored the association between SMD and BA.

The liver plays an important role in the aging process through metabolism[20]. Abnormal levels of two common enzymes in the liver, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may induce hepatocellular damage and consequently lead to DNA damage, which is considered one of the important factors in cellular aging[21]. Additionally, evidence suggests a positive association between obesity-related indicators-BMI and SMD[11], with obesity also being regarded as a disease that accelerates biological aging[22]. Therefore, BMI and AST/ALT were considered as possible mediators of the association between SMD and biological age in our study.

To fill this gap, we performed a cross-sectional investigation in a well-established cohort of 71,579 adults in the United Kingdom to examine the association of SMD with two forms of AgeAccel, including KDMAccel and PhenoAgeAccel.

2. Methods and materials

2.1 Study population

Data from 500K individuals in the UK Biobank were available in our dataset. The UK Biobank, a large-scale biomedical cohort study, collected comprehensive health information from nearly 0.5 million participants aged 37 to 73 years across the United Kingdom[23]. From 2006 to 2010, participants completed a face-to-face interview with professionals, a touch screen questionnaire, and a whole-body physical examination and donated biological samples, including blood, urine, and saliva at the nearest one of 22 assessment centers. All participants provided written informed consent. Participants who met certain criteria were excluded from

this study. These criteria included missing dietary information (n=293,373), missing BA information (n=65,784), non-white and genetically related participants (n=31,615), and missing values of covariates (n=40,188). After exclusion, the final analyses included 71,579 individuals (Supplemental Figure S1). The UK Biobank study had been approved by the North West Multi-Centre Research Ethics Committee (reference number 06/MRE09/65).

2.2 Assessment of the Sulfur Microbial Diet score

The Oxford WebQ utilized in the UK Biobank is an online 24-hour diet recall tool comprising questions on the consumption of nearly 200 foods and drinks. Its validity has been established through biomarker validation studies conducted elsewhere[24]. Participants were asked to complete the questionnaire on five separate occasions between 2009 and 2012, considering the seasonal variation in diet. Subsequently, an average measure was calculated for each participant across all five occasions to mitigate measurement error bias. These occasions included the baseline assessment (April 2009–September 2010, 70,684 participants), online cycle 1 (February 2011–April 2011, 100,574 participants), online cycle 2 (June 2011–September 2011, 83,239 participants), online cycle 3 (October 2011–December 2011, 103,761 participants), and online cycle 4 (April 2012–June 2012, 100,219 participants). In our study, 209,166 participants with complete dietary information were included after excluding those with missing data. For each participant, the average intake of each food item was calculated based on up to five dietary recall surveys.

Previous studies have specifically described the calculation methodology of SMD score[7]. In brief, prior investigations identified 43 sulfur-metabolizing bacterial species carrying genes coding for at least two well-known sulfur-metabolizing enzymes. A reduced rank regression was performed to link food intake with the log-transformed abundance of these microbes in stool. The analysis identified eight food groups significantly associated with sulfur-metabolizing bacteria. The finding was further validated in subsequent research and was found to explain 2% of the variation in Bray–Curtis distances[25; 26]. Specifically, certain food groups such as processed meats, liquor, and low-calorie drinks were positively associated with these bacteria, while others like beer, fruit juice, legumes, vegetables, and sweets/desserts were negatively associated. Based on the intake of these foods, the SMD

score was calculated by summing the product of beta-coefficients and corresponding serving sizes (Supplement Table S1). A higher SMD score reflects closer adherence to this dietary pattern, and is considered 'unhealthy'.

2.3 Assessment of the Biological Age

In this study, BA values were calculated using KDM and PhenoAge methods based on participants from the UK Biobank. The two methods had different purposes. KDM was calculated by performing a series of regressions on the biomarkers of CA, to quantify the decline in system integrity[27]. PhenoAge was calculated based on biomarkers and mortality prediction scores and CA and is used to predict the risk of death[27].

In brief, KDM was calculated from forced expiratory volume in one second (FEV1), systolic blood pressure, and seven blood chemistry parameters (albumin, alkaline phosphatase, blood urea nitrogen, creatinine, C-reactive protein, glycated hemoglobin, and total cholesterol); PhenoAge was calculated based on nine blood chemistries (albumin, alkaline phosphatase, creatinine, C-reactive protein, glucose, mean cell volume, red cell distribution width, white blood cell count, and lymphocyte proportion), four of which were the same as KDM. The BA values were calculated using the R package "BioAge" for KDM and PhenoAge. The residual of the regression of BA on chronological age is used to reflect the age acceleration (AgeAccel), referred to as "KDMAccel" and "PhenoAgeAccel"[28]. AgeAccel serves as the target outcome in our analysis. Participants with AgeAccel values greater than 0 were considered to have accelerated biological aging[19]. And based on whether AgeAccel was greater than 0, participants were classified into a binary variable, termed biological age indicators. More information was shown in the Supplement information.

2.4 Assessment of covariates

To control for potential confounding variables, we included covariates such as age, sex, body mass index (BMI), townsend deprivation index (TDI), education, income, smoke frequency per day, alcohol frequency per week, hypertension, diabetes, and coronary heart disease (CHD), which may be associated with aging, as well as dietary factors. At the baseline assessment center visit, a trained nurse measured the participants' height and weight, and BMI was calculated by dividing weight in kilograms by the square of height in meters. TDI

was an indicator of material deprivation that was calculated based on non-home ownership, non-car ownership, unemployment, and household overcrowding. A higher TDI score indicates higher levels of deprivation. Smoke frequency was defined as the maximum number of reported past or current cigarettes (or pipes/cigars) consumed per day. Alcohol frequency per week was determined as the average amount of various types of alcohol consumed per week. Education was defined by the UK Biobank and converted to the International Standard Classification of Education (ISCED). Income was categorized into five groups (≤ £18,000, 18,000-30,999, 31,000-51,999, 52,000-100,000, and ≥ 100,000). Self-reported information and medical records were used to determine the history of hypertension, CHD, and diabetes. Observations with missing values for any covariate were excluded from this study. Supplement information provides detailed measurements of covariates.

2.5 Statistical analyses

In the present analysis, the SMD score was categorized into quartiles, and the lowest quartile was considered as the reference category. Values of baseline characteristics across the quartiles of the SMD score were indicated as the means ± SD for continuous variables or percentages (%) for categorical variables, respectively. We initially used generalized linear regression models to investigate the associations between the SMD score quartiles and two forms of AgeAccel and estimate β-coefficient and standard error (SE), where AgeAccel served as the outcome variable. The regression analysis was conducted by three models: Model 1 adjusted for age and sex; Model 2 included additional adjustments for BMI, TDI, education, income, smoke frequency per day, and alcohol frequency per week; Model 3 further incorporated hypertension, diabetes, and CHD. Multiple stratified analyses were applied to evaluate the possible modifying effects of the following factors: age (≥ 60 / < 60 years), sex (male/female), smoke status (yes/no), and BMI (≤ 24.9 / 24.9 to 29.9 / ≥ 30 kg/m^2). Interaction analysis between the stratifying variables and SMD score was tested. The dose-response curves between the SMD score with AgeAccel and biological age indicators were analyzed by restricted cubic spline (RCS) regression. Mediation effect analyses were used to assess associations of SMD score with AgeAccel mediated by BMI and serum AST/ALT ratio.

All statistical analyses were completed through the software R 4.2.1, and statistical significance was determined by a two-sided *P*-value threshold of less than 0.05.

3. Results

3.1 Baseline characteristics

The descriptive statistics of participants are presented in Table 1. Among the 71,579 participants from the UK Biobank, the mean age was 56.04 ± 7.81 years, with 51.32% being women at baseline. Participants with a higher SMD score tended to be younger, have a higher household income and BMI, a higher frequency of smoke and alcohol consumption, a higher proportion of major diseases, and a higher degree of AgeAccel compared to those with lower adherence.

3.2 Associations between SMD score quartiles and AgeAccel

As shown in Figure 1 and Table 2, adherence to SMD was significantly associated with the two forms of AgeAccel ($P_{\text{-trend}} < 0.05$). After adjusting for all covariates, compared with the first quartile of SMD score, β (*95%CI*) of KDMAccel was 0.16 (0.07, 0.24; $P<0.001$) for the second quartile, 0.21 (0.13, 0.29; $P<0.001$) for the third quartile, and 0.35 (0.27, 0.44; $P<0.001$) for the highest quartile. Meanwhile, β (*95%CI*) of PhenoAgeAccel was 0.07 (-0.01, 0.16; $P = 0.100$) for the second quartile, 0.18 (0.09, 0.27; $P<0.001$) for the third quartile, and 0.32 (0.23, 0.41; $P<0.001$) for the highest quartile.

3.3 Association of SMD score quartiles with AgeAccel stratified by baseline characteristics

Similar associations were detected while conducting extensive stratified analyses based on the variables of age, sex, smoke status, and BMI levels. Notably, in Model 3, compared to females (KDMAccel: $\beta_{\text{Q4vsQ1}} = 0.21$, *95%CI*: 0.13-0.30, $P<0.001$; PhenoAgeAccel: $\beta_{\text{Q4vsQ1}} = 0.31$, *95%CI*: 0.19-0.44, $P<0.001$) and non-smokers (KDMAccel: $\beta_{\text{Q4vsQ1}} = 0.31$, *95%CI*: 0.21-0.41, $P<0.001$; PhenoAgeAccel: $\beta_{\text{Q4vsQ1}} = 0.19$, *95%CI*: 0.08-0.30, $P<0.001$), the association between SMD quartiles and AgeAccel exhibited greater strength among males (KDMAccel: $\beta_{\text{Q4vsQ1}} = 0.51$, *95%CI*: 0.37-0.65, $P<0.001$; PhenoAgeAccel: $\beta_{\text{Q4vsQ1}} = 0.37$, *95%CI*: 0.25-0.49, $P<0.001$) and smokers (KDMAccel: $\beta_{\text{Q4vsQ1}} = 0.46$, *95%CI*: 0.32-0.61, $P<0.001$; PhenoAgeAccel: $\beta_{\text{Q4vsQ1}} = 0.58$, *95%CI*: 0.42-0.73, $P<0.001$). More detailed

information is shown in Figure 1.

3.4 Best-fitting dose-response curves of the associations of SMD score with AgeAccel and biological age indicators

For SMD score, the linearities and dose-response associations with AgeAccel and age indicators were flexibly modeled by conducting RCS regression models (Figure 2). After multivariable adjustment, monotonic and linear associations were observed between SMD and KDMAccel ($P_{overall} < 0.001$, $P_{nonlinearity} = 0.821$), SMD and KDM biological age indicator ($P_{overall} = 0.001$, $P_{nonlinearity} = 0.951$), SMD and PhenoAgeAccel ($P_{overall} < 0.001$, $P_{nonlinearity} = 0.265$), and SMD and PhenoAge biological age indicator ($P_{overall} =0.021$, $P_{nonlinearity} = 0.550$).

3.5 Mediation effects of BMI and AST/ALT ratio on the associations of SMD score with AgeAccel

Mediation statistical models were performed to ascertain whether BMI and serum AST/ALT ratio had mediation effects on the associations. After adjustment for all the covariables in the linear regression model, the total effect of SMD score on KDMAccel and PhenoAgeAccel were 0.079 ($P = 9.10\times10^{-21}$) and 0.078 ($P = 1.05\times10^{-17}$) (Table 2). For the SMD score, the indirect effects on KDMAccel and PhenoAgeAccel were mediated by BMI measured at 0.049 (38.02%) and 0.040 (34.17%). The indirect effects on KDMAccel and PhenoAgeAccel mediated by AST/ALT measured at 0.004 (5.49%) and -0.005 (6.29%). More information is shown in Figure 3.

4. Discussion

In this cross-sectional study involving 71,579 participants, we observed a significant positive association between a dietary pattern linked to a higher SMD score and an elevation of AgeAccel, as assessed by multiple markers using two widely accepted algorithms. Furthermore, a higher score of SMD was associated with an increase in AgeAccel among males and smokers. Both BMI and serum AST/ALT ratio mediated the association between SMD and two forms of AgeAccel. These findings support the hypothesis that dietary variations with lower detrimental microbiome configurations related to sulfur metabolism may be associated with lower biological aging.

The SMD is characterized by a low intake of fruits, vegetables, legumes, whole grains, and nuts, along with a high intake of red meat, processed meat products, high-fat dairy products, sugary beverages, and coffee[29]. However, Wang et al. indicated that the relative abundance of sulfur-metabolizing bacteria may be determined by dietary sources of sulfur and specific sulfur-containing compounds, rather than total sulfur content[25]. As for food components, red and processed meats are rich in both sulfur-containing amino acids and inorganic sulfur from preservatives[25], and also can significantly worsen age-related diseases, such as cardiovascular disease, sarcopenia, cognitive dysfunction, and cancer[30]. In addition, a study utilizing plasma protein profiles to identify accelerated and decelerated aging discovered that the consumption of sugar-sweetened beverages increased the predicted chronological age by 2-6 years[31]. On the contrary, a high intake of whole grains, vegetables, fruits, and nuts is associated with a reduced risk for all-cause mortality[32]. Cruciferous vegetables are rich in sulfur-containing glucosinolates, which can be hydrolyzed to isothiocyanates by gut microbiota that express myrosinase. Isothiocyanates and their downstream products have anticarcinogenic effects[33]. What's more, healthy dietary patterns such as Mediterranean, Japanese, Okinawan, and Nordic diets have been associated with long-term survival and a reduced incidence of non-communicable diseases[34]. These dietary patterns share common features that may explain the mechanisms of healthy aging. For example, increase the intake of vegetables and whole grain foods, consume unsaturated fatty acids, have a moderate intake of protein, primarily plant-based, avoid or limit alcohol consumption, avoid red meat and processed meats, and limit sugar intake[34].

Our results were biologically possible via microbial H_2S generation and extended previous findings demonstrating the role of diet-induced microbial changes in the aging process. Current evidence indicates that persistent low-grade inflammation and oxidative stress accelerate cellular and tissue aging, which has adverse effects on biological age[35]. Inflammation and oxidative stress are vital physiological processes influenced by various factors such as age, diet, and lifestyle[19]. H_2S is produced within the gastrointestinal tract by resident gut bacteria metabolizing sulfates, sulfites, and various proteins[36]. H_2S exhibits genotoxicity, promoting inflammation and causing DNA damage in epithelial cells. Elevated

concentrations of H_2S disrupt intestinal permeability, facilitating the absorption of lipopolysaccharides into the bloodstream, further exacerbating inflammation[37], which is associated with increased risks of obesity[11], non-alcoholic fatty liver disease[10], and gastrointestinal cancers[38], thereby increasing the risk of aging.

In our study, we found that the association between SMD and AgeAccel is partially mediated by BMI and AST/ALT ratio, which are biomarkers of obesity status and liver function. Atypical elevation of AST/ALT is used to assess liver function and the severity of liver disease, suggesting the presence of underlying liver issues[39]. Liver dysfunction can impact aging, primarily manifested as declining liver function, malnutrition, hormonal imbalance, and immune dysregulation[40]. Obesity leads to many adverse health outcomes, including cardiovascular diseases, diabetes, and cancer, which are considered age-related diseases[41]. Meanwhile, it is significantly positively associated with epigenetic AgeAccel[42]. Therefore, these two indicators are considered potential risk factors for the association. Moreover, the mediation results suggest that reducing body weight and supporting liver health by adherence to dietary pattern with lower SMD score may help slow down the aging process.

It is worth noting that a more significant association between SMD quartiles and AgeAccel in males and smoking populations was observed. There are several possible explanations for this observation. Firstly, adherence to the SMD diet is positively associated with the risk of obesity, with sex stratification revealing a more significant positive association in males than females[11]. Secondly, there is a higher proportion of males among smokers in our study. Smoking is associated with increased levels of inflammatory oxidative stress[19]. Specifically, smoking damages intestinal barrier function, promotes inflammatory responses in the process of intestinal disease occurrence, and enhances carcinogenic MAPK/ERK signaling[43], as evidenced by a study finding a positive correlation between SMD and the risk of colorectal adenomas, particularly notable in males and smokers[38].

The present study contains some strengths. Firstly, our study includes a relatively large sample size and rich information on dietary pattern. Secondly, previous studies have rarely examined the effect of the sulfur-metabolism microbial diet on biological aging. We were the

first to assess the effect of BMI and AST/ALT ratio as a mediator on biological aging. Nonetheless, limitations should be acknowledged. Firstly, a substantial number of participants were excluded due to missing dietary or BA information, which may introduce selection bias and limit generalizability. However, the large sample size and rigorous methodology help mitigate these concerns. Secondly, it is worth noting that the sulfur microbial diet was initially constructed based on a US cohort of older men. However, the UK Biobank does not provide information on the microbiomes of the participants, which prevents us from evaluating the effect estimates for specific food types. Thirdly, limited by the cross-sectional study design, we are unable to assume causality of the observed association. Fourthly, although the 24-hour diet was retrospectively assessed several times at baseline, this may have allowed participants to change their dietary patterns. Finally, as our study was conducted with a predominantly White sample of 37-73 years participants, caution is needed when generalizing our findings to other populations. Future analyses comparing SMD with other dietary patterns in terms of shared components and health outcomes, as well as exploring the potential interactions between systemic inflammation or metabolic pathways and sulfur-metabolizing bacteria would be valuable.

In conclusion, this study is the first to discover the accelerated effects of a gut microbiota-derived dietary pattern, the sulfur microbial dietary pattern, on biological aging. The monotonic and linear association between them emphasized the change beyond the threshold in the sulfur bacterial diet score dramatically increasing the risk of AgeAccel. BMI and serum AST/ALT mediate the association between SMD and two AgeAccel. These findings support not only the role of diet in the aging process but also the possibility of using a gut microbiota-targeted dietary modification to slow down the aging process.

Statements and Declarations

Ethics approval: The North West Multi-Centre Research Ethics Committee (MREC) and Human Tissue Authority (HTA) have approved UK Biobank.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for publication: Informed consent was obtained from all subjects involved in the study.

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Author Contributions: Y.L. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. F.Z. conceptualized and designed the study. All authors contributed to the data's acquisition, analysis, and interpretation. Y.L. drafted the manuscript. F.Z. helped with the critical revision of the manuscript for important intellectual content. Y.L. performed the statistical analysis. F.Z. provided administrative, technical, or material support. F.Z. supervised the study. All authors have read and agreed to the published version of the manuscript.

Competing Interests: All authors report no biomedical financial interests or potential conflicts of interest.

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Table 1. Basic characteristics of participants from UK Biobank according to the sulfur microbial diet score.

Characteristics	Quartile of sulfur microbial diet score [c]			
	Q1	Q2	Q3	Q4
N	17,890	17,894	17,819	17,976
Male (%)	8,583 (47.98%)	8,497 (47.49%)	8,464 (47.50%)	9,299 (51.73%)
Age (year)	56.5 (7.64)	56.46 (7.74)	56.08 (7.82)	55.13 (7.95)
BMI (kg/m^2)	26.37 (4.30)	26.56 (4.28)	26.73 (4.30)	27.54 (4.64)
TDI	-1.81 (2.67)	-1.92 (2.65)	-1.90 (2.66)	-1.76 (2.76)
Smoke frequency per day	5.95 (9.97)	5.55 (9.59)	5.63 (9.75)	6.39 (10.38)
Alcohol frequency per week	9.89 (9.25)	9.77 (8.92)	9.76 (9.33)	10.51 (10.39)
Household income (£) (%)				
<18,000	2,516 (14.06%)	2,365 (13.22%)	2,418 (13.57%)	2,441 (13.58%)
18,000 to 30,999	4,496 (25.13%)	4,295 (24.00%)	4,213 (23.64%)	4,096 (22.79%)
31,000 to 51,999	5,177 (28.94%)	5,260 (29.40%)	5,169 (29.01%)	5,243 (29.17%)
52,000 to 100,000	4,423 (24.72%)	4,603 (25.72%)	4,626 (25.96%)	4,781 (26.60%)
>100,000	1,278 (7.14%)	1,371 (7.66%)	1,393 (7.82%)	1,415 (7.87%)
Major diseases				
Diabetes (%)	911 (5.09%)	930 (5.20%)	984 (5.52%)	1,327 (7.38%)
Hypertension (%)	4,684 (26.18%)	4,642 (25.94%)	4,655 (26.12%)	5,000 (27.81%)
Coronary heart disease (%)	1,600 (8.94%)	1,595 (8.91%)	1,637 (9.19%)	1,788 (9.95%)
Sulfur microbial diet score	-4.41 (1.14)	-2.50 (0.35)	-1.43 (0.28)	-0.01 (0.85)
Biological age				
KDM biological age (year)	50.25 (9.12)	50.45 (9.19)	50.20 (9.24)	49.41 (9.30)
KDMAccel	-6.24 (5.37)	-6.01 (5.33)	-5.88 (5.29)	-5.71 (5.29)
PhenoAge (year)	47.40 (9.00)	47.45 (9.06)	47.23 (9.17)	46.70 (9.41)
PhenoAgeAccel	-9.10 (4.47)	-9.01 (4.41)	-8.85 (4.45)	-8.43 (4.63)
Components of biological ages				

FEV_1(L) [a]	2.89 (0.76)	2.86 (0.76)	2.86 (0.77)	2.90 (0.77)
SBP (mm Hg) [a]	137.66 (18.25)	137.38 (18.22)	136.85 (17.93)	136.88 (17.78)
Total cholesterol (mg/dL) [a]	222.34 (42.59)	222.50 (42.57)	222.61 (42.92)	221.24 (43.49)
Glycated hemoglobin (%) [a]	3.51 (0.51)	3.52 (0.53)	3.52 (0.54)	3.55 (0.62)
Blood urea nitrogen (mg/dL) [a]	14.83 (3.58)	14.98 (3.50)	15.02 (3.54)	15.18 (3.66)
Lymphocyte (%) [b]	28.92 (7.33)	29.01 (7.27)	28.89 (7.20)	28.81 (7.21)
Mean cell volume (fL) [b]	83.22 (5.20)	83.11 (5.18)	83.08 (5.21)	83.01 (5.26)
Serum glucose (mg/dL) [b]	91.26 (17.28)	91.44 (18.00)	91.44 (18.00)	91.98 (20.16)
Red cell distribution width (%) [b]	13.41 (0.87)	13.41 (0.89)	13.42 (0.89)	13.43 (0.90)
White blood cell count (1000 cells/μl) [b]	6.66 (2.01)	6.69 (1.71)	6.73 (1.83)	6.84 (1.73)
Albumin (g/dL) [a,b]	4.56 (0.26)	4.55 (0.26)	4.55 (0.26)	4.55 (0.26)
Creatinine (mg/dL) [a,b]	0.79 (0.16)	0.79 (0.16)	0.80 (0.16)	0.81 (0.18)
C-reactive protein (mg/dL) [a,b]	0.43 (0.22)	0.43 (0.22)	0.44 (0.23)	0.46 (0.24)
Alkaline phosphatase (U/L) [a,b]	80.20 (22.57)	80.41 (24.93)	80.83 (24.47)	80.85 (24.41)

Note: Data are either percentage or mean ± SD unless indicated otherwise. BMI, body mass index; TDI, Townsend deprivation index; KDM, Klemera-Doubal method; KDMAccel, KDM biological age acceleration; PhenoAgeAccel, PhenoAge acceleration. [a] Components of KDM biological age. [b] Components of PhenoAge. [c] The sulfur microbial diet score was calculated by summing the intake of foods (processed meats, liquor, low-calorie drinks, beer, fruit juice, legumes, other vegetables, sweets, and desserts) weighted by their regression coefficients (β).

Table 2. Multivariable-adjusted associations between the sulfur microbial diet score and AgeAccel.

	Quartiles of the sulfur microbial diet score							*P*-trend [a]	Per 1-SD	
	Q1	Q2	*P*	Q3	*P*	Q4	*P*		β (SE)	*P*
KDMAccel										
Model 1	Reference	0.196 (0.045)	**1.21×10^{-5}**	0.327 (0.045)	**3.01×10^{-13}**	0.763 (0.045)	**8.88×10^{-65}**	**4.21×10^{-65}**	0.165 (0.009)	**2.25×10^{-75}**
Model 2	Reference	0.151 (0.042)	**3.70×10^{-4}**	0.212 (0.042)	**5.63×10^{-7}**	0.370 (0.043)	**4.28×10^{-18}**	**4.09×10^{-18}**	0.083 (0.009)	**4.07×10^{-22}**
Model 3	Reference	0.156 (0.042)	**2.00×10^{-4}**	0.212 (0.042)	**4.42×10^{-7}**	0.353 (0.042)	**7.41×10^{-17}**	**8.09×10^{-17}**	0.079 (0.008)	**9.10×10^{-21}**
PhenoAgeAccel										
Model 1	Reference	0.096 (0.047)	**0.040**	0.265 (0.047)	**1.69×10^{-8}**	0.647 (0.047)	**3.73×10^{-43}**	**1.01×10^{-45}**	0.146 (0.009)	**8.99×10^{-55}**
Model 2	Reference	0.073 (0.045)	0.109	0.186 (0.045)	**4.04×10^{-5}**	0.343 (0.046)	**4.93×10^{-14}**	**2.26×10^{-15}**	0.083 (0.009)	**7.35×10^{-20}**
Model 3	Reference	0.074 (0.045)	0.101	0.181 (0.045)	**5.69×10^{-5}**	0.317 (0.045)	**2.87×10^{-12}**	**1.71×10^{-13}**	0.078 (0.009)	**1.05×10^{-17}**

Note: For KDMAccel and PhenoAgeAccel, the effect was shown by coefficient (β) and standard error (*SE*).

[a] Tests for trend were conducted using the quartile category as a continuous variable.

Model 1 was adjusted for age and sex.

Model 2 was further adjusted for BMI, TDI, education, income, smoke frequency per day, and alcohol frequency per week.

Model 3 was further adjusted for hypertension, diabetes, and coronary heart diseases.

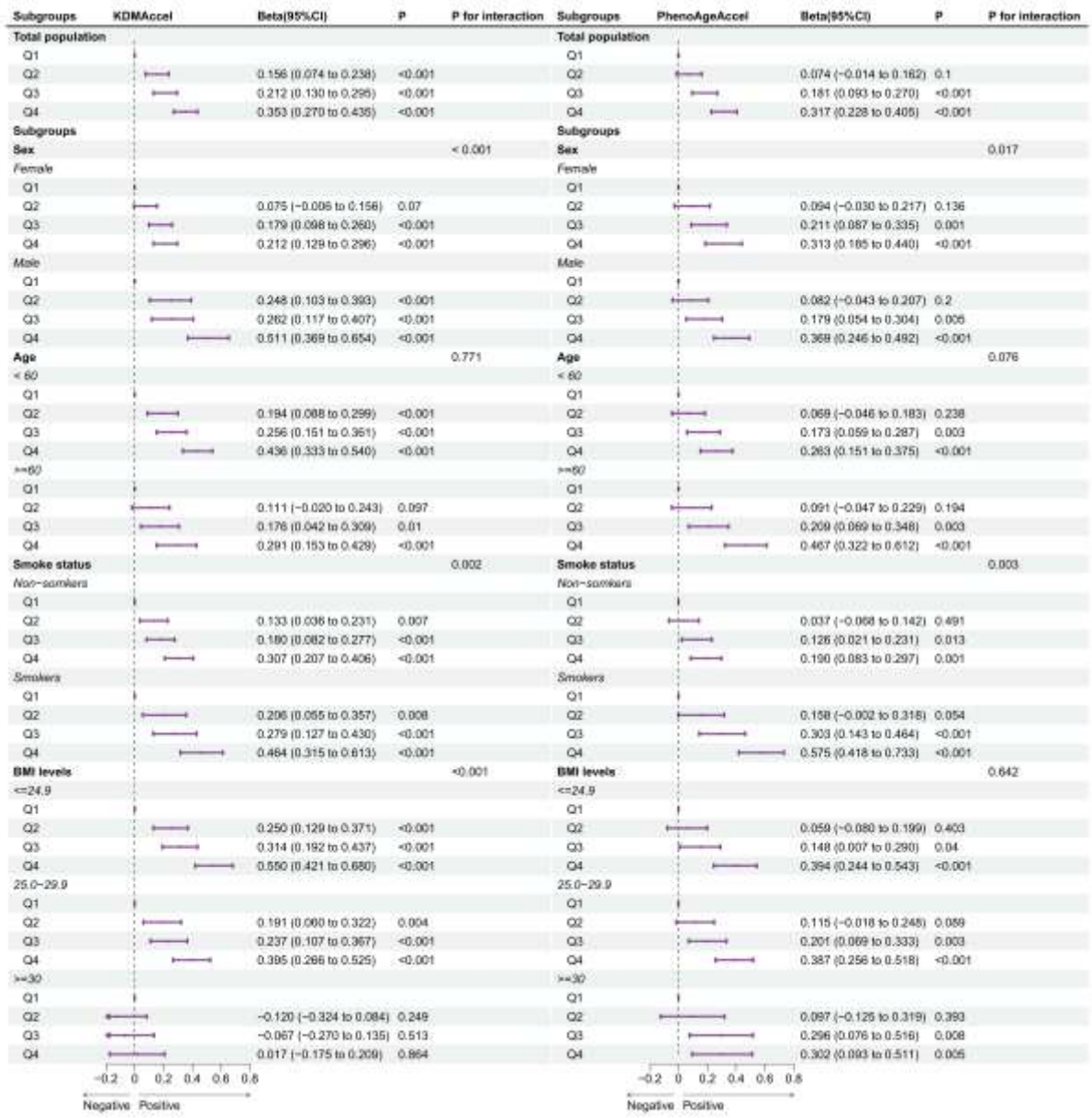

Figure 1. Forest plot of the association of the sulfur microbial diet score quartiles with two forms of age acceleration and its subgroup analyses. The adjustments involved the covariables selected in the full regression model. Q, quartiles; CI, confidence intervals; BMI, body mass index.

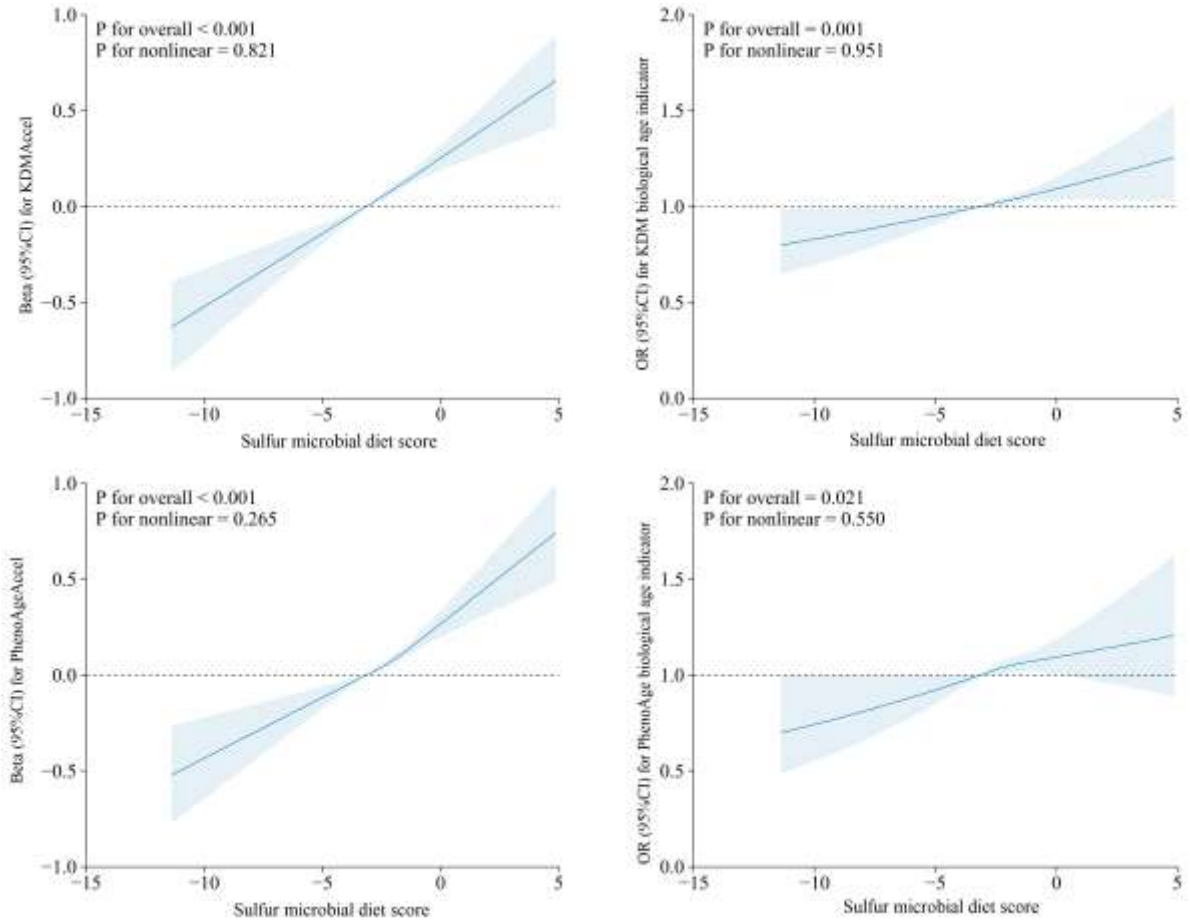

Figure 2. Association of the sulfur microbial diet score with age acceleration and biological age indicators evaluated by linear and binomial logistic regression models and RCS after adjusted for all covariates. The solid blue lines correspond to the central estimate, and the blue -shaded regions indicate the 95% confidence intervals.

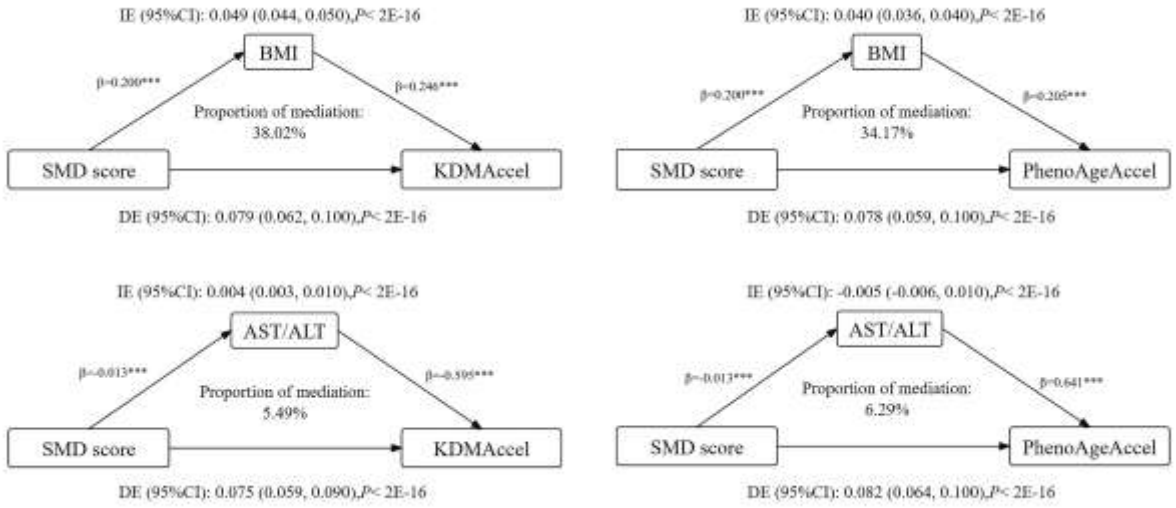

Figure 3. Effects mediated by BMI and serum AST/ALT ratio on the associations of the sulfur microbial diet score with two forms of AgeAccel. IE, indirect effect; DE, direct effect. ***$P < 0.001$.